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(54) Title: CDR-GRAFTED ANTI-TISSUE FACTOR ANTIBODIES AND METHODS OF USE THEREOF

(57) Abstract

The present invention provides CDR-grafted antibodies against human tissue factor that retain the high binding affinity of rodent monoclonal antibodies against tissue factor but have reduced immunogenicity. The present humanized antibodies are potent anticoagulants and are thus useful in the treatment and prophylaxis of human thrombotic disease. The invention also provides methods of making the CDR-grafted antibodies and pharmaceutical compositions for the attenuation or prevention of coagulation.

CDR-GRAFTED ANTI-TISSUE FACTOR
1   ANTIBODIES AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

5   Monoclonal antibodies capable of inhibiting
tissue factor (TF) are useful as anticoagulants.
Conventional rodent monoclonal antibodies, however, have
limited use in human therapeutic and diagnostic
applications due to immunogenicity and short serum half-
10 life. The present invention provides CDR-grafted
monoclonal antibodies against TF that retain the high
binding affinity of rodent antibodies but have reduced
immunogenicity. The present humanized antibodies are
potent anticoagulants and are thus useful in the
15 treatment and prophylaxis of human thrombotic disease.
The invention also provides methods of making the CDR-
grafted antibodies and pharmaceutical compositions for
the attenuation or prevention of coagulation.

BACKGROUND OF THE INVENTION
20

            The coagulation of blood involves a cascading
series of reactions leading to the formation of fibrin.
The coagulation cascade consists of two overlapping
25 pathways, both of which are required for hemostasis.
The intrinsic pathway comprises protein factors present
in circulating blood, while the extrinsic pathway
requires tissue factor, which is expressed on the cell
surface of a variety of tissues in response to vascular
30 injury. Davie et al., 1991, Biochemistry 30:10363.
Agents that interfere with the coagulation cascade, such

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as heparin and coumarin derivatives, have well-known
1 therapeutic uses in the prophylaxis of venous
thrombosis. Goodman and Gilman, eds., 1980, The
Pharmacological Basis of Therapeutics, MacMillan
Publishing Co., Inc., New York.
5   Tissue factor (TF) has been investigated as a
target for anticoagulant therapy. TF is a membrane
glycoprotein that functions as a receptor for factor VII
and VIIa and thereby initiates the extrinsic pathway of
the coagulation cascade in response to vascular injury.
10 In addition to its role in the maintenance of hemostasis
by initiation of blood clotting, TF has been implicated
in pathogenic conditions. Specifically, the synthesis
and cell surface expression of TF has been implicated in
vascular disease (Wilcox et al., 1989, Proc. Natl. Acad.
15 Sci. 86:2839) and gram-negative septic shock (Warr et
al., 1990, Blood 75:1481).
           Ruf et al. (1991, Thrombosis and Haemostasis
66:529) characterized the anticoagulant potential of
murine monoclonal antibodies against human TF. The
20 inhibition of TF function by most of the monoclonal
antibodies that were assessed was dependent upon the
dissociation of the TF/VIIa complex that is rapidly
formed when TF contacts plasma. Such antibodies were
thus relatively slow inhibitors of TF in plasma. One
25 monoclonal antibody, TF8-5G9, was capable of inhibiting
the TF/VIIa complex without dissociation of the complex,
thus providing an immediate anticoagulant effect in
plasma. Ruf et al. suggest that mechanisms that
inactivate the TF/VIIa complex, rather than prevent its
30 formation, may provide strategies for interruption of
coagulation in vivo.

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                  The therapeutic use of monoclonal antibodies
1 against TF is limited in that currently available
   monoclonals are of rodent origin. The use of rodent
   antibodies in human therapy presents numerous problems,
   the most significant of which is immunogenicity.
5 Repeated doses of rodent monoclonal antibodies have been
   found to elicit an anti-immunoglobulin response termed
   human anti-mouse antibody (HAMA), which can result in
   immune complex disease and/or neutralization of the
   therapeutic antibody. See, e.g., Jaffers et al. (1986)
10 Transplantation 41:572. While the use of human
   monoclonal antibodies would address this limitation, it
   has proven difficult to generate large amounts of human
   monoclonal antibodies by conventional hybridoma
   technology.
15   Recombinant technology has been used in an
   effort to construct "humanized" antibodies that maintain
   the high binding affinity of rodent monoclonal
   antibodies but exhibit reduced immunogenicity in humans.
   Chimeric antibodies have been produced in which the
20 variable (V) region of a mouse antibody is combined with
   the constant (C) region of a human antibody in an effort
   to maintain the specificity and affinity of the rodent
   antibody but reduce the amount of protein that is non-
   human and thus immunogenic. While the immune response
25 to chimeric antibodies is generally reduced relative to
   the corresponding rodent antibody, the immune response
   cannot be completely eliminated, because the mouse V
   region is capable of eliciting an immune response.
   Lobuglio et al. (1989) Proc. Natl. Acad. Sci. 86:4220;
30 Jaffers et al. (1986) Transplantation 41:572.

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In a recent approach to reducing
1 immunogenicity of rodent antibodies, only the rodent
complementarity determining regions (CDRs), rather than
the entire V domain, are transplanted to a human
antibody. Such humanized antibodies are known as CDR-
5 grafted antibodies. CDRs are regions of
hypervariability in the V regions that are flanked by
relatively conserved regions known as framework (FR)
regions. Each V domain contains three CDRs flanked by
four FRs. The CDRs fold to form the antigen binding
10 site of the antibody, while the FRs support the
structural conformations of the V domains. Thus by
transplanting the rodent CDRs to a human antibody, the
antigen binding domain can theoretically also be
transferred. Owens et al. (1994) J. Immunol. Methods
15 168:149 and Winter et al. (1993) Immunology Today 14:243
review the development of CDR-grafted antibodies.
Orlandi et al. (1989) Proc. Natl. Acad. Sci.
USA 86:3833 constructed a humanized antibody against the
relatively simple hapten nitrophenacetyl (NP). The CDR-
20 grafted antibody contained mouse CDRs and human FRs, and
exhibited NP binding activity similar to the native
mouse antibody. However, the construction of CDR-
grafted antibodies recognizing more complex antigens has
resulted in antibodies having binding activity
25 significantly lower than the native rodent antibodies.
In numerous cases it has been demonstrated that the mere
introduction of rodent CDRs into a human antibody
background is insufficient to maintain full binding
activity, perhaps due to distortion of the CDR
30 conformation by the human FR.

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        For example, Gorman et al. (1991) Proc. Natl.
1   Acad. Sci. 88:4181 compared two humanized antibodies
    against human CD4 and observed considerably different
    avidies depending upon the particular human framework
    region of the humanized antibody. Co et al. (1991)
5   Proc. Natl. Acad. Sci. USA 88:2869 required a refined
    computer model of the murine antibody of interest in
    order to identify critical amino acids to be considered
    in the design of a humanized antibody. Kettleborough et
    al. (1991) Protein Engineering 4:773 report the
10 influence of particular FR residues of a CDR-grafted
    antibody on antigen binding, and propose that the
    residues may directly interact with antigen, or may
    alter the conformation of the CDR loops. Similarly,
    Singer et al. (1993) J. Immunol. 150:2844 report that
15 optimal humanization of an anti-CD18 murine monoclonal
    antibody is dependent upon the ability of the selected
    FR to support the CDR in the appropriate antigen binding
    conformation. Accordingly, recreation of the antigen-
    binding site requires consideration of the potential
20 intrachain interactions between the FR and CDR, and
    manipulation of amino acid residues of the FR that
    maintain contacts with the loops formed by the CDRs.
    While general theoretical guidelines have been proposed
    for the design of humanized antibodies (see, e.g., Owens
25 et al.), in all cases the procedure must be tailored and
    optimized for the particular rodent antibody of
    interest.

        There is a need in the art for humanized
    antibodies with reduced immunogenicity and comparable
30 binding affinity relative to the parent rodent antibody
    for various therapeutic applications. In particular,

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there is a need for a humanized antibody against human

1 tissue factor having anticoagulant activity and useful
in the treatment and prevention of thrombotic disease.

SUMMARY OF THE INVENTION

5

The present invention is directed to CDR-
grafted antibodies capable of inhibiting human tissue
factor wherein the CDRs are derived from a non-human
monoclonal antibody against tissue factor and the FR and

10 constant (C) regions are derived from one or more human
antibodies. In a preferred embodiment, the murine
monoclonal antibody is TF8-5G9.

In another embodiment, the present invention
provides a method of producing a CDR-grafted antibody

15 capable of inhibiting human tissue factor which method
comprises constructing one or more expression vectors
containing nucleic acids encoding CDR-grafted antibody
heavy and light chains, transfecting suitable host cells
with the expression vector or vectors, culturing the

20 transfected host cells, and recovering the CDR-grafted
antibody.

The present invention also provides a method
of attenuation of coagulation comprising administering a
CDR-grafted antibody capable of inhibiting human tissue

25 factor to a patient in need of such attenuation.
The present invention further provides a
method of treatment or prevention of thrombotic disease
comprising administering a CDR-grafted antibody capable
of inhibiting human tissue factor to a patient in need

30 of such treatment or prevention. In a preferred

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embodiment, the thrombotic disease is intravascular
1   coagulation, arterial restenosis or arteriosclerosis.
        Another embodiment of the present invention is
    directed to a pharmaceutical composition comprising CDR-
    grafted antibodies capable of inhibiting human tissue
5   factor and further comprising a pharmaceutically
    acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

10   Fig. 1 provides the nucleotide and deduced
    amino acid sequences of the heavy chain of murine
    monoclonal antibody TF8-5G9.
        Fig. 2 provides the nucleotide and deduced
    amino acid sequences of the light chain of murine
15 monoclonal antibody TF8-5G9.
        Fig. 3 is a graph depicting the ability of
    CDR-grafted antibody TF8HCDR1 x TF8LCDR1 to bind to
    human tissue factor and to compete with murine
    monoclonal antibody TF85G9 for binding to tissue factor.
20 Solid symbols indicate direct binding of TF8HCDR1 x
    TF8LCDR1 and the positive control chimeric TF85G9 to
    tissue factor. Open symbols indicate competition
    binding of TF8HCDR1 x TF8LCDR1 or chimeric TF85G9 with
    murine monoclonal antibody TF85G9.
25   Fig. 4 presents the DNA sequence of expression
    vector pEe6TF8HCDR20 and the amino acid sequence of the
    coding regions of the CDR-grafted heavy chain TF8HCDR20.
        Fig. 5 presents the DNA sequence of expression
    vector pEe12TF8LCDR3 and the amino acid sequence of the
30 coding regions of the CDR-grafted light chain TF8LCDR3.

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Fig. 6 is a graph depicting the ability of
1 CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to bind to
human tissue factor.

Fig. 7 is a graph depicting the ability of
CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to compete
5 with murine monoclonal antibody TF85G9 for binding to
tissue factor.

Fig. 8 is a graph depicting the ability of
CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to inhibit
factor X activation.

10   Fig. 9 provides expression vector
pEe6TF8HCDR20 resulting from the subcloning of CDR-
grafted heavy chain TF8HCDR20 into myeloma expression
vector pEehCMV-BglI. The following abbreviations are
used: VH is the CDR-grafted heavy chain variable
15 region; Cγ4 is the human IgG4 constant region; pA is the
polyadenylation signal; ampR is the ß-lactamase gene;
and hCMV is human cytomegalovirus.

Fig. 10 provides expression vector
pEe12TF8LCDR3 resulting from the subcloning of CDR-
20 grafted light chain TF8LCDR3 into myeloma expression
vector pEe12. The following abbreviations are used: VL
is the CDR-grafted light chain variable region; CK is
the human kappa constant region; SVE is the SV40 early
promoter; GS is glutamine synthetase cDNA. Other
25 abbreviations are as noted in Fig. 9.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides CDR-grafted
30 antibodies capable of inhibiting human tissue factor
wherein the CDRs are derived from a non-human monoclonal

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antibody against tissue factor and the FR and C regions
1 are derived from one or more human antibodies. The
   present invention further provides methods of making and
   using the subject CDR-grafted antibodies.

        In accordance with the present invention, the
5 CDR-grafted antibody is an antibody in which the CDRs
   are derived from a non-human antibody capable of binding
   to and inhibiting the function of human tissue factor,
   and the FR and C regions of the antibody are derived
   from one or more human antibodies. The CDRs derived
10 from the non-human antibody preferably have from about
   90% to about 100% identity with the CDRs of the non-
   human antibody, although any and all modifications,
   including substitutions, insertions and deletions, are
   contemplated so long as the CDR-grafted antibody
15 maintains the ability to bind to and inhibit tissue
   factor. The regions of the CDR-grafted antibodies that
   are derived from human antibodies need not have 100%
   identity with the human antibodies. In a preferred
   embodiment, as many of the human amino acid residues as
20 possible are retained in order than immunogenicity is
   negligible, but the human residues, in particular
   residues of the FR region, are substituted as required
   and as taught hereinbelow in accordance with the present
   invention. Such modifications as disclosed herein are
25 necessary to support the antigen binding site formed by
   the CDRs while simultaneously maximizing the
   humanization of the antibody.

        Non-human monoclonal antibodies against human
   tissue factor from which the CDRs can be derived are
30 known in the art (Ruf et al., 1991; Morrisey et al.,
   1988, Thrombosis Research 52:247) or can be produced by

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well-known methods of monoclonal antibody production

1   (see, e.g. Harlow et al., eds., 1988, Antibodies, A
    Laboratory Manual, Cold Spring Harbor Laboratories, Cold
    Spring Harbor, New York). Purified human tissue factor
    against which monoclonal antibodies can be raised is
5   similarly well-known (Morrisey et al., 1987, Cell
    50:129) and available to the skilled artisan. Murine
    monoclonal antibodies, and in particular murine
    monoclonal antibody TF8-5G9 disclosed by Ruf et al. and
    Morrisey et al., 1988, Thrombosis Research 52:247, and
10 U.S. Patent No. 5,223,427 are particularly preferred.
        The ordinarily skilled artisan can determine
    the sequences of the CDRs by reference to published
    scientific literature or sequence databanks, or by
    cloning and sequencing the heavy and light chains of the
15 antibodies by conventional methodology. In accordance
    with the present invention, the cDNA and amino acid
    sequences of the heavy chain (SEQ ID NOS:1 and 2,
    respectively) and light chain (SEQ ID NOS:3 and 4,
    respectively) of murine monoclonal antibody TF8-5G9 are
20 provided. The cDNA and deduced amino acid sequence of
    the murine TF8-5G9 heavy chain is provided at Figure 1.
    The cDNA and deduced amino acid sequence of the murine
    TF8-5G9 light chain is provided at Figure 2.
        Each of the heavy and light chain variable
25 regions contain three CDRs that combine to form the
    antigen binding site. The three CDRs are surrounded by
    four FR regions that primarily function to support the
    CDRs. The sequences of the CDRs within the sequences of
    the variable regions of the heavy and light chains can
30 be identified by computer-assisted alignment according
    to Kabat et al. (1987) in Sequences of Proteins of

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<u>Immunological Interest</u>, 4th ed., United States
1 Department of Health and Human Services, US Government
   Printing Office, Washington, D.C., or by molecular
   modeling of the variable regions, for example utilizing
   the ENCAD program as described by Levitt (1983) <u>J. Mol.</u>
5 <u>Biol.</u> <u>168</u>:595.

               -          In a preferred embodiment the CDRs are derived
   from murine monoclonal antibody TF8-5G9. The preferred
   heavy chain CDRs have the following sequences:

10   CDR1   DDYMH   (SEQ ID NO:5)
                CDR2   LIDPENGNTIYDPKFQG   (SEQ ID NO:6)
                CDR3   DNSYYFDY   (SEQ ID NO:7)

   The preferred light chain CDRs have the following
15 sequences:

                CDR1   KASQDIRKYLN   (SEQ ID NO:8)
                CDR2   YATSLAD   (SEQ ID NO:9)
                CDR3   LQHGESPYT   (SEQ ID NO:10)
20

   The sequences of the CDRs of the murine or other non-
   human antibody, and in particular the sequences of the
   CDRs of TF8-5G9, may be modified by insertions,
   substitutions and deletions to the extent that the CDR-
25 grafted antibody maintains the ability to bind to and
   inhibit human tissue factor. The ordinarily skilled
   artisan can ascertain the maintenance of this activity
   by performing the functional assays described
   hereinbelow. The CDRs can have, for example, from about
30 50% to about 100% homology to the CDRs of SEQ ID NOS:5-
   10. In a preferred embodiment the CDRs have from about

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80% to about 100% homology to the CDRs of SEQ ID NOS:5-
1   10. In a more preferred embodiment the CDRs have from
     about 90% to about 100% homology to the CDRs of SEQ ID
     NOS:5-10. In a most preferred embodiment the CDRs have
     from about 100% homology to the CDRs of SEQ ID NOS:5-10.
5   The FR and C regions of the CDR-grafted
     antibodies of the present invention are derived from one
     or more human antibodies. Human antibodies of the same
     class and type as the antibody from which the CDRs are
     derived are preferred. The FR of the variable region of
10   the heavy chain is preferably derived from the human
     antibody KOL (Schmidt et al., 1983, Hoppe-Seyler's Z.
     Physiol. Chem. 364:713) The FR of the variable region
     of the light chain is preferably derived from the human
     antibody REI (Epp et al., 1974, Eur. J. Biochem.
15   45:513). In accordance with the present invention, it
     has been discovered that certain residues of the human
     FR are preferably replaced by the corresponding residue
     of the non-human antibody from which the CDRs are
     derived. For example, certain FR residues of TF8-5G9
20   are preferably retained to achieve optimal binding to
     antigen.
             For convenience, the numbering scheme of Kabat
     et al. has been adopted herein. Residues are designated
     by lower case numbers or hyphens as necessary to conform
25   the present sequences to the standard Kabat numbered
     sequence.
             In accordance with the present invention,
     residues that are retained in the FR region, i.e
     residues that are not replaced by human FR residues, are
30   determined according to the following guidelines.
     Residues that are idiosyncratic to the parent antibody,

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e.g. TF8-5G9, relative to a human consensus sequence of
1   Kabat et al, are retained. Residues of the parent
    antibody that are in agreement with the consensus
    sequence are retained if the corresponding residue of
    the human antibody, e.g. KOL or REI, is idiosyncratic.
5   Residues that are part of the antibody loop canonical
    structures defined by Chothia et al. (1989) Nature
    342:877, such as residue 71 of the heavy and light
    chains, are retained. FR residues predicted to form
    loops, such as residues 28-30 of the heavy chain, are
10 retained. FR residues predicted to influence the
    conformation of the CDRs such as residues 48 and 49
    preceding CDR2 of the heavy chain, are retained.
    Residues that have been demonstrated to be critical in
    the humanization of other antibodies may also be
15 retained. The foregoing guidelines are followed to the
    extent necessary to support the antigen binding site
    formed by the CDRs while simultaneously maximizing the
    humanization of the antibody.
            The amino acid sequence of a representative
20 CDR-grafted heavy chain variable region derived from
    murine monoclonal antibody TF8-5G9 and human antibody
    KOL is shown below. The CDR-grafted heavy chain is
    designated TF8HCDR1; murine residues were retained in
    the FR at residues 6, 17, 23, 24, 28, 29, 30, 48, 49,
25 68, 71, 73, 78, 88 and 91. CDRs are underlined.

```
            10          20          30     35ab            50
QVQLVQSGGG VVQPGRLLRL SCKASGFNIK DYYMH--WVR QAPGKGLEWIG
52abc       60          70              80 82abc       90
LIDP--ENGNTIYD PKFQGRFSIS ADTSK--NTAFL QMDSLRPEDTAVY
100         110
```
30 YCARDNSYYF DYWGQGTPVT VSS (SEQ ID NO:11)

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The amino acid sequence of a representative
1 CDR-grafted light chain variable region derived from
murine monoclonal antibody TF8-5G9 and human antibody
REI is shown below. The CDR-grafted light chain is
designated TF8LCDR1; murine residues were retained in
5 the FR at residues 39, 41, 46 and 105. CDRs are
underlined.


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           10        20        30        40        50
      DIQMTQSPSS LSASVGDRVT ITCKASQDIR KYLNWYQQK WKAPKTLIYY
10         60        70        80        90        100
      ATSLADGVPS RFSGSGSGTD YTFTISSLQP EDIATYYCLQ HGESPYTFGQ

      GTKLEITR  (SEQ ID NO:12)
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A CDR-grafted antibody containing variable
15 regions TF8HCDR1 and TF8LCDR1 has been demonstrated in
accordance with the present invention to be as effective
as murine monoclonal antibody TF8-5G9 in binding to
human tissue factor. It has been further discovered in
accordance with the present invention, by examination of
20 the molecular structure of murine monoclonal antibody
TF8-5G9, and by design, construction, and analysis of
CDR-grafted antibodies, that the FR regions can be
further humanized without the loss of antigen binding
activity. In particular, the FR region may retain the
25 human FR residue at residues 6, 17, 68, 73 and 78 of the
heavy chain, and residues 39, 41, 16 and 105 of the
light chain, with maintenance of antigen binding
activity.
In a most preferred embodiment, the heavy
30 chain variable region contains a FR derived from human
antibody KOL in which murine monoclonal antibody TF8-5G9

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residues are retained at amino acids 23, 24, 28, 29, 30,
1   48, 49, 71, 88 and 91. The preferred heavy chain
    variable region is designated TF8HCDR20 and has the
    following sequence.

5   10   20   30   35ab   50
       QVQLVESGGG VVQPGRSLRL SCKASGFNIK DYYMH--WVR QAPGKGLEWIGL

       52abc   60   70   80 82abc   90   100
       IDP--ENGNTIYD PKFQGRFTIS ADNSKNTLFL QMDSLRPEDTAVY YCARDNSYYF

10   110
       DYWGQGTPVT VSS   (SEQ ID NO:13)

            In a most preferred embodiment, the light
       chain variable region contains a FR derived from human
15   antibody REI in which murine monoclonal antibody TF8-5G9
       residues are retained at amino acids 39 and 105. The
       preferred light chain variable region is designated
       TF8LCDR20 and has the following sequence.
               10   20   30   40   50
        DIQMTQSPSS LSASVGDRVT ITCKASQDIR KYLNWYQQKP GKAPKLLIYY
20   60   70   80   90   100
       ATSLADGVPS RFSGSGSGTD YTFTISSLQP EDIATYYCLQ HGESPYTFGQ
       GTKLEITR (SEQ ID NO:14)

            It is within the ken of the ordinarily skilled
25   artisan to make minor modifications of the foregoing
       sequences, including amino acid substitutions, deletions
       and insertions. Any such modifications are within the
       scope of the present invention so long as the resulting
       CDR-grafted antibody maintains the ability to bind to
30   and inhibit human tissue factor. The ordinarily skilled
       artisan can assess the activity of the CDR-grafted

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antibody with reference to the functional assays
1 described hereinbelow.

The human constant region of the CDR-grafted
antibodies of the present invention is selected to
minimize effector function. The intended use of the
5 CDR-grafted antibodies of the present invention is to
block the coagulation cascade by inhibition of tissue
factor, and thus antibody effector functions such as
fixation of complement are not desirable. Antibodies
with minimal effector functions include IgG2, IgG4, IgA,
10 IgD and IgE. In a preferred embodiment of the present
invention, the heavy chain constant region is the human
IgG4 constant region, and the light chain constant
region is the human IgG4 kappa constant region.

In that effector functions may not be
15 desirable for therapeutic uses, the present invention
further contemplates active fragments of the CDR-grafted
antibodies, and in particular Fab fragments and $F(ab')_2$
fragments. Active fragments are those fragments capable
of inhibiting human tissue factor. Fab fragments and
20 $F(ab')_2$ fragments may be obtained by conventional means,
for example by cleavage of the CDR-grafted antibodies of
the invention with an appropriate proteolytic enzyme
such as papain or pepsin, or by recombinant production.
The active fragments maintain the antigen binding sites
25 of the CDR-grafted antibodies and thus are similarly
useful therapeutically.

The ability of the CDR-grafted antibodies
designed and constructed as taught in accordance with
the present invention to bind and inhibit human tissue
30 factor can be assessed by functional assays. For
example, in a rapid and convenient assay, expression

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vectors containing nucleic acids encoding the CDR-
1 grafted heavy and light chains can be co-transfected
into suitable host cells and transiently expressed. The
resulting antibodies can be assessed by standard assays
for ability to bind human tissue factor, and for ability
5 to compete for binding to tissue factor with the non-
human antibody from which the CDRs are derived.

For example, transient expression of nucleic
acids encoding the CDR-grafted heavy and light chains in
COS cells provides a rapid and convenient system to test
10 antibody gene expression and function. Nucleic acids
encoding the CDR-grafted heavy and light chains,
respectively, are cloned into a mammalian cell
expression vector, for example pSG5, described by Green
et al. (1988) Nucleic Acids Res. 16:369 and commercially
15 available from Stratagene Cloning Systems, La Jolla, CA.
The pSG5 expression vector provides unique restriction
sites for the insertion of the heavy and light chain
genes, and in vivo expression is under the control of
the SV40 early promoter. Transcriptional termination is
20 signaled by the SV40 polyadenylation signal sequence.

The pSG5-based expression vectors containing
nucleic acids encoding the heavy and light chains are
cotransfected into COS cells and cultured under
conditions suitable for transient expression. Cell
25 culture media is then harvested and examined for
antibody expression, for example by an enzyme linked
immunosorbent assay (ELISA), to determine that suitable
levels of antibody have been produced. An ELISA may
then be used to assess the ability of the CDR-grafted
30 antibody to bind to human tissue factor. Human tissue
factor is immobilized on a microtiter plate and the COS

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cell supernatant containing the CDR-grafted antibody is
1   added followed by an incubation at room temperature for
    about one hour. The plates are then washed with a
    suitable detergent-containing buffer such as phosphate
    buffered saline (PBS)/Tween, followed by the addition of
5   the components of a suitable detection system. For
    example, horseradish peroxidase conjugated goat anti-
    human kappa chain polyclonal antibody is added, followed
    by washing, followed by addition of substrate for
    horseradish peroxidase, and detection. The CDR-grafted
10 antibodies within the scope of the present invention are
    those which are capable of binding to human tissue
    factor to a degree comparable to the non-human antibody
    from which the CDRs are derived as determined by the
    foregoing assay.
15   The ability of the CDR-grafted antibodies to
    inhibit the activity of human tissue factor _in vivo_ can
    be conveniently assessed by the following _in vitro_ assay
    that mimics _in vivo_ coagulation events. In response to
    vascular injury _in vivo_, tissue factor binds to factor
20 VII and facilitates the conversion of factor VII to a
    serine protease (factor VIIa). The factor VIIa-tissue
    factor complex converts factor X to a serine protease
    (factor Xa). Factor Xa forms a complex with factor Va
    (from the intrinsic coagulation pathway), resulting in
25 the conversion of prothrombin to thrombin, which in turn
    results in the conversion of fibrinogen to fibrin. In a
    convenient _in vitro_ functional assay, tissue factor is
    incubated in the presence of factor VIIa and the CDR-
    grafted anti-tissue factor antibody produced in the
30 transient expression system described above. Factor X
    is added and the reaction mixture is incubated, followed

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by an assay for factor Xa activity utilizing a

1   chromogenic substrate for factor Xa (Spectrozyme FXa,
American Diagnostica, Inc., Greenwich, CT). The ability
of the CDR-grafted antibody to inhibit factor X
activation thus provides a measure of the ability of the

5   CDR-grafted antibody to inhibit the activity of human
tissue factor.

The CDR-grafted antibodies within the scope of
the present invention are those which are capable of
inhibiting human tissue factor to a degree comparable to

10 the non-human antibody from which the CDRs are derived
as determined by the foregoing assay. In one
embodiment, the CDR-grafted antibody has at least 50% of
the inhibitory activity of TF8-5G9 for human tissue
factor. In a preferred embodiment, the CDR-grafted

15 antibody has at least 70% of the inhibitory activity of
TF8-5G9 for human tissue factor. In a more preferred
embodiment, the CDR-grafted antibody has at least 80% of
the inhibitory activity of TF8-5G9 for human tissue
factor. In a most preferred embodiment, the CDR-grafted

20 antibody has at least 90% of the inhibitory activity of
TF8-5G9 for human tissue factor.

In another embodiment, the present invention
provides a method of producing a CDR-grafted antibody
capable of inhibiting human tissue factor. The method

25 comprises constructing an expression vector containing a
nucleic acid encoding the CDR-grafted antibody heavy
chain and an expression vector containing a nucleic acid
encoding the CDR-grafted antibody light chain,
transfecting suitable host cells with the expression

30 vectors, culturing the transfected host cells under
conditions suitable for the expression of the heavy and

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light chains, and recovering the CDR-grafted antibody.
1 Alternately, one expression vector containing nucleic
   acids encoding the heavy and light chains may be
   utilized.

        Standard molecular biological techniques, for
5 example as disclosed by Sambrook et al. (1989),
   Molecular Cloning: A Laboratory Manual Cold Spring
   Harbor Press, Cold Spring Harbor, NY may be used to
   obtain nucleic acids encoding the heavy and light chains
   of the CDR-grafted antibodies of the present invention.
10 A nucleic acid encoding the CDR-grafted variable domain
   may be constructed by isolating cDNA encoding the
   antibody to be humanized, e.g. murine monoclonal
   antibody TF8-5G9, by conventional cloning methodology
   from the hybridoma producing the antibody, or by
15 polymerase chain reaction (PCR) amplification of the
   variable region genes, as described for example by
   Winter et al., followed by site-directed mutagenesis to
   substitute nucleotides encoding the desired human
   residues into the FR regions. Alternately, the cDNA
20 encoding the human antibody can be isolated, followed by
   site-directed mutagenesis to substitute nucleotides
   encoding the desired murine residues into the CDRs.
        Nucleic acids encoding the CDR-grafted
   variable domain may also be synthesized by assembling
25 synthetic oligonucleotides, for example utilizing DNA
   polymerase and DNA ligase. The resulting synthetic
   variable regions may then be amplified by PCR. Nucleic
   acids encoding CDR-grafted variable domains may also be
   constructed by PCR strand overlap methods that are known
30 in the art and reviewed by Owens et al.

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Accordingly, having determined the desired

1 amino acid sequences of the CDR-grafted variable domains in accordance with the present invention, the ordinarily skilled artisan can obtain nucleic acids encoding the variable domains. Further, the skilled artisan is aware

5 that due to the degeneracy of the genetic code, various nucleic acid sequences can be constructed that encode the CDR-grafted variable domains. All such nucleic acid sequence are contemplated by the present invention.

The nucleic acids encoding the CDR-grafted

10 variable domains are linked to appropriate nucleic acids encoding the human antibody heavy or light chain constant region. Nucleic acid sequences encoding human heavy and light chain constant regions are known in the art. It is within the ken of the ordinarily skilled

15 artisan to include sequences that facilitate transcription, translation and secretion, for example start codons, leader sequences, the Kozak consensus sequence (Kozak, 1987, J. Mol. Biol. 196:947) and the like, as well as restriction endonuclease sites to

20 facilitate cloning into expression vectors.

The present invention thus further provides nucleic acids encoding the heavy and light chains of CDR-grafted antibodies capable of inhibiting human tissue factor wherein the CDRs are derived from a murine

25 monoclonal antibody against tissue factor and the FR and C regions are derived from one or more human antibodies.

In accordance with the present invention, representative nucleic acids encoding CDR-grafted heavy and light chains were constructed. The CDR-grafted

30 heavy chain comprises a variable region containing FR regions derived from human antibody KOL and CDRs derived

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                  from murine monoclonal antibody TF8-5G9 and further
1   comprises a constant region derived from the heavy chain
      of human IgG4. The CDR-grafted light chain comprises a
      variable region containing FR regions derived from human
      antibody REI and CDRs derived from murine monoclonal
5   antibody TF8-5G9 and further comprises a constant region
      derived from human IgG4 kappa chain. Nucleic acids
      encoding the heavy and light chains were constructed by
      assembling the variable regions from synthetic
      nucleotides, amplifying the assembled variable regions
10   by PCR, purifying the amplified nucleic acids, and
      ligating the nucleic acid encoding the variable region
      into a vector containing a nucleic acid encoding the
      appropriate human constant region.
                  The sequences of representative nucleic acids
15   encoding CDR-grafted heavy and light chains are
      presented as nucleotides 1-2360 of SEQ ID NO:15 and
      nucleotides 1-759 of SEQ ID NO:20, respectively.
                  The nucleic acid sequence encoding a preferred
      heavy chain (nucleotides 1-2360 of SEQ ID NO:15) is
20   designated the TF8HCDR20 gene. The nucleic acid
      sequence contains the following regions: 5' EcoRI
      restriction site (nucleotides 1-6); Kozak sequence
      (nucleotides 7-15); start codon and leader sequence
      (nucleotides 16-72); CDR-grafted variable region
25   (nucleotides 73-423); human IgG4 CH1 domain (nucleotides
      424-717); human IgG4 intron 2 (nucleotides 718-1110);
      human IgG4 hinge (nucleotides 1111-1146); human IgG4
      intron 3 (nucleotides 1147-1267); human IgG4 CH2 domain
      (nucleotides 1268-1594); human IgG4 intron 4
30   (nucleotides 1595-1691); human IgG4 CH3 domain
      (nucleotides 1692-2012); 3' untranslated region

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(nucleotides 2013-2354); 3' BamHI end spliced to BclI
1 site of expression vector (nucleotides 2355-2360).

The nucleic acid sequence encoding a preferred
light chain gene (nucleotides 1-759 of SEQ ID NO:20) is
designated the TF8LCDR3 gene. The nucleic acid sequence
5 contains the following regions: 5' EcoRI restriction
site- (nucleotides 1-5); Kozak sequence (nucleotides 6-
8); start codon and leader sequence (nucleotides 9-68);
CDR-grafted variable region (nucleotides 69-392); human
kappa constant region (nucleotides 393-710); 3'
10 untranslated region (nucleotides 711-753); 3' BamHI end
spliced to BclI site of expression vector (nucleotides
754-759).

The foregoing preferred sequences can be
modified by the ordinarily skilled artisan to take into
15 account degeneracy of the genetic code, and to make
additions, deletions, and conservative and
nonconservative substitutions that result in a
maintenance of the function of the nucleic acid, i.e.
that it encodes a heavy or light chain of a CDR-grafted
20 antibody capable of inhibiting human tissue factor.
Restriction sites and sequences that facilitate
transcription and translation may be altered or
substituted as necessary depending upon the vector and
host system chosen for expression.
25   Suitable expression vectors and hosts for
production of the CDR-grafted antibodies of the present
invention are known to the ordinarily skilled artisan.
The expression vectors contain regulatory sequences,
such as replicons and promoters, capable of directing
30 replication and expression of heterologous nucleic acids
sequences in a particular host cell. The vectors may

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also contain selection genes, enhancers, signal
1   sequences, ribosome binding sites, RNA splice sites,
    polyadenylation sites, transcriptional terminator
    sequences, and so on. The vectors may be constructed by
    conventional methods well-known in the art, or obtained
5   from commercial sources. The expression vectors
    preferably have convenient restriction sites at which
    the nucleic acids encoding the antibody chains of the
    invention are inserted. Myeloma expression vectors in
    which antibody gene expression is driven by the human
10 cytomegalovirus promoter-enhancer or are particularly
    preferred.

         Expression vectors containing a nucleic acid
    encoding the CDR-grafted heavy chain under the control
    of a suitable promoter and expression vectors containing
15 a nucleic acid encoding the CDR-grafted light chain
    under the control of a suitable promoter are
    cotransfected into a suitable host cell. In another
    embodiment, nucleic acids encoding both heavy and light
    chains are provided in a single vector for transfection
20 of a suitable host cell.

         Suitable host cells or cell lines for
    expression of the CDR-grafted antibodies of the present
    invention include bacterial cells, yeast cells, insect
    cells, and mammalian cells such as Chinese hamster ovary
25 (CHO) cells, COS cells, fibroblast cells and myeloid
    cells. Mammalian cells are preferred. CHO, COS and
    myeloma cells are particularly preferred. Myeloma cells
    are preferred for establishing permanent CDR-grafted
    antibody producing cell lines. Expression of antibodies
30 in myeloma cells, bacteria, and yeast is reviewed by

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Sandhu (1992) <u>Critical Reviews in Biotechnology</u> 12:437.

1   Expression in mammalian cells is reviewed by Owen <u>et</u> <u>al</u>..
Transfection of host cells by the expression
vectors containing nucleic acids encoding the CDR-
grafted heavy and light chains can be accomplished by
5   methods well-known to one of ordinary skill in the art.
Such methods include, for example, calcium chloride
transfection, calcium phosphate transfection,
lipofection and electroporation. Suitable culture
methods and conditions for the production of the CDR-
10 grafted antibodies are likewise well-known in the art.
The CDR-grafted antibodies can be purified by
conventional methods, including ammonium sulfate
precipitation, affinity chromatography, gel
electrophoresis, and the like. The ability of the CDR-
15 grafted antibodies to bind to and inhibit human tissue
factor can be assessed by the <u>in vitro</u> assays described
above.

The CDR-grafted antibodies of the present
invention have a variety of utilities. For example, the
20 antibodies are capable of binding to human tissue factor
and thus are useful in assays for human tissue factor
from body fluid samples, purification of human tissue
factor, and so on.

The CDR-grafted antibodies of the present
25 invention are capable of inhibiting human tissue factor.
Human tissue factor is well-known to be an essential
element in the human coagulation cascade. The ability
of the antibodies of the present invention to disrupt
the coagulation cascade is demonstrated by <u>in vitro</u>
30 assays in which the antibodies prevent factor X
activation. Accordingly, the present antibodies are

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useful in the attenuation of coagulation. The present
1 invention thus provides a method of attenuation of
coagulation comprising administering a therapeutically
effective amount of CDR-grafted antibody capable of
inhibiting human tissue factor to a patient in need of
5 such attenuation.

Numerous thrombotic disorders are
characterized by excessive or inappropriate coagulation
and are effectively treated or prevented by
administration of agents that interfere with the
10 coagulation cascade. Accordingly, the present invention
further provides a method of treatment or prevention of
a thrombotic disorder comprising administering a
therapeutically effective amount of a CDR-grafted
antibody capable of inhibiting human tissue factor to a
15 patient in need of such treatment or prevention. In a
preferred embodiment, the thrombotic disorder is
intravascular coagulation, arterial restenosis or
arteriosclerosis. The antibodies of the invention may be
used in combination with other antibodies or therapeutic
20 agents.

A therapeutically effective amount of the
antibodies of the present invention can be determined by
the ordinarily skilled artisan with regard to the
patient's condition, the condition being treated, the
25 method of administration, and so on. A therapeutically
effective amount is the dosage necessary to alleviate,
eliminate, or prevent the thrombotic disorder as
assessed by conventional parameters. For example, a
therapeutically effective dose of a CDR-grafted antibody
30 of the present invention may be from about 0.1 mg to
about 20 mg per 70 kg of body weight. A preferred

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dosage is about 1.0 mg to about 5 mg per 70 kg of body
1 weight.

A patient in need of such treatment is a
patient suffering from a disorder characterized by
inappropriate or excessive coagulation, or a patient at
5 risk of such a disorder. For example, anticoagulant
therapy is useful to prevent postoperative venous
thrombosis, and arterial restenosis following balloon
angioplasty.

The CDR-grafted antibodies of the present
10 invention are useful in the same manner as comparable
therapeutic agents, and the dosage level is of the same
order of magnitude as is generally employed with those
comparable therapeutic agents. The present antibodies
may be administered in combination with a
15 pharmaceutically acceptable carrier by methods known to
one of ordinary skill in the art.

Another embodiment of the present invention is
directed to a pharmaceutical composition comprising a
least one CDR-grafted antibody capable of inhibiting
20 human tissue factor and further comprising a
pharmaceutically acceptable carrier. As used herein,
"pharmaceutically acceptable carrier" includes any and
all solvents, dispersion media, coatings, antibacterial
and antifungal agents, isotonic and absorption delaying
25 agents, and the like. The use of such media and agents
for pharmaceutically active substances is well-known in
the art. Except insofar as any conventional media or
agent is incompatible with the active ingredient, its
use in the therapeutic compositions is contemplated.
30 Supplementary active ingredients can also be
incorporated into the compositions.

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The antibodies can be administered by well-
1 known routes including oral and parenteral, e.g.,
intravenous, intramuscular, intranasal, intradermal,
subcutaneous, and the like. Parenteral administration
and particularly intravenous administration is
5 preferred. Depending on the route of administration,
the pharmaceutical composition may require protective
coatings.

The pharmaceutical forms suitable for
injectionable use include sterile aqueous solutions or
10 dispersions and sterile powders for the extemporaneous
preparation of sterile injectable solutions or
dispersions. In all cases the ultimate solution form
must be sterile and fluid. Typical carriers include a
solvent or dispersion medium containing, for example,
15 water buffered aqueous solutions (i.e., biocompatible
buffers), ethanol, polyol such as glycerol, propylene
glycol, polyethylene glycol, suitable mixtures thereof,
surfactants or vegetable oils. The antibodies may be
incorporated into liposomes for parenteral
20 administration. Sterilization can be accomplished by an
art-recognized techniques, including but not limited to,
addition of antibacterial or antifungal agents, for
example, paraben, chlorobutanol, phenol, sorbic acid or
thimersal. Further, isotonic agents such as sugars or
25 sodium chloride may be incorporated in the subject
compositions.

Production of sterile injectable solutions
containing the subject antibodies is accomplished by
incorporating these antibodies in the required amount in
30 the appropriate solvent with various ingredients
enumerated above, as required, followed by

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sterilization, preferably filter sterilization. To

1 obtain a sterile powder, the above solutions are vacuum-dried or freeze-dried as necessary.

The following examples further illustrate the present invention.

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EXAMPLE 1

1   Isolation and Sequencing of TF8-5G9
              Light Chain (LC) and Heavy Chain (HC)

              Two DNA libraries were generated from oligo
5   (dT)-primed TF8-5G9 hybridoma RNA utilizing standard
    molecular biology procedures as described by Sambrook et
    al. The cDNA was cloned into the Librarian II plasmid
    vector from Invitrogen (San Diego, CA), and the
    libraries were screened for cDNA clones encoding murine
10 IgG HC and LC. A full-length cDNA clone for the heavy
    chain could not be isolated, despite the construction of
    two independent libraries. A random primed TF8-5G9 cDNA
    library was generated to obtain the missing 5' sequence
    of the heavy chain. Consequently, the heavy chain cDNA
15 was in two pieces: a 5' clone of 390 nucleotides and a
    3' clone of 1392 nucleotides. The two HC clones overlap
    by 292 nucleotides.
              The HC and LC clones were completely sequenced
    by the dideoxy chain termination method of Sanger et al.
20 (1977) Proc. Natl. Acad. Sci. USA 74:5463. To verify
    the variable region sequence, sequence was obtained from
    PCR-amplified cDNA that had been synthesized from total
    TF8-5G9 hybridoma RNA. Total TF8-5G9 hybridoma RNA was
    isolated by the guanidinium thiocyanate method of
25 Chrigwin et al. (1970) Biochemistry 18:5294. cDNA was
    synthesized using the Perkin Elmer (Norwalk, CT) GeneAmp
    RNA Polymerase Chain Reaction (PCR) kit with an oligo
    (dT) primer. Components of the same kit were used in
    the PCR to amplify the LC and HC variable regions using
30 primers based on the sequence that had been obtained for
    the cDNA clones. The amplified variable region

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fragments were gel-purified and sequenced according to
1   the method of Tracy et al. (1991) BioTechniques 11:68 on
     a Model 373A Applied Biosystems, Inc. (Foster City, CA)
     automated fluorescent DNA sequencer. The sequence for
     TF8-5G9 LC and HC obtained from RNA amplification and
5   the sequence obtained from the cDNA clones agreed. The
     TF8-5G9 HC variable region sequence with protein
     translation is shown in Figure 1 and SEQ ID NO:1, and
     that for the LC is shown in Figure 2 and SEQ ID NO:3.

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EXAMPLE 2

1   Chimeric LC and HC Expression Vector Construction

              In order to test the binding activity of the
      CDR-grafted anti-TF LC and HC individually, mouse-human
5   chimeric TF8-5G9 LC and HC were constructed. This
      allowed the CDR-grafted LC to be tested for TF binding
      ability in combination with the chimeric HC, and the
      CDR-grafted HC to be tested in combination with the
      chimeric LC.
10   Primers were designed to amplify the TF8-5G9
      LC variable region using as template cDNA clones in the
      Librarian II vector. The 5' primer was designed with an
      EcoRI site while the 3' primer was designed with a NarI
      site. PCR was used to amplify the LC variable region,
15   generating a 433 bp fragment with a 5'EcoRI end and
      3'NarI end. The fragment included the signal sequence
      from the TF8-5G9 LC cDNA clone but incorporated a 2 base
      change in the arginine codon immediately following the
      ATG start codon. This change retained the arginine
20   residue but made the sequence conform to the Kozak
      consensus sequence in order to potentially improve
      translation of the LC mRNA. The PCR amplified LC
      variable region fragment was digested with EcoRI and
      NarI restriction enzymes and purified by electrophoresis
25   on a 2% Nusieve, 1% Seakem agarose gel (FMC Bio
      Products, Rockland, ME).
              The DNA was extracted from the gel slice and
      purified by the Geneclean (Bio 101, La Jolla, CA)
      procedure. The full-length chimeric TF8-5G9 LC gene was
30   generated by cloning this DNA into the EcoRI and NarI
      sites of a pSP73 vector (Promega, Madison, WI) which

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contains the human kappa constant region. The gene was
1 isolated from the pSP73 vector by EcoRI digestion and
   subcloned into the EcoRI site of the pSG5 mammalian cell
   expression vector (Stratagene Cloning Systems, La Jolla,
   CA).

5   The chimeric TF8-5G9 HC gene was assembled in
   a manner similar to that of the chimeric LC. Since
   there was no full-length HC cDNA isolated from the
   Librarian II vector cDNA libraries, the HC variable
   region fragment that was generated by the PCR from total
10 TF8-5G9 hybridoma cell RNA was used as the template.
   Primers which incorporated an EcoRI site at the 5' end
   and a SacI site at the 3' end were used in the PCR to
   generate a 430 bp fragment which contained the TF8-5G9
   HC Kozak sequence, start codon, signal sequence, and
15 variable region. This fragment was digested with the
   restriction enzymes EcoRI and SacI, and gel-purified
   using the same procedure that was used with the chimeric
   LC construction.

           The full-length TF8-5G9 chimeric HC gene was
20 constructed by cloning the variable region fragment into
   the EcoRI and SacI sites of the pSG5 expression vector
   containing the human IgG4 constant region.

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EXAMPLE 3

1   Design and Construction of the
                CDR-Grafted Heavy and Light Chain Genes

              The variable region domains of the CDR-grafted
5   HC and LC genes were designed with an EcoRI overhang at
     the 5' end followed by a Kozak sequence to improve
     antibody expression. The leader sequences were derived
     from the heavy and light chains of the murine monoclonal
     antibody B72.3 (Whittle et al. (1987) Protein
10   Engineering 1:499). The 3' end of the variable regions
     were designed to have overhangs which allowed for
     splicing to the appropriate human constant region DNA.
              In the initially designed CDR-grafted TF8-5G9
     heavy and light chains the CDRs were derived from murine
15   TF8-5G9 sequence while the frameworks were derived
     primarily from human antibody sequence. The human
     antibody KOL (Schmidt et al.) was used for the heavy
     chain frameworks, while the human antibody dimer (Epp et
     al.) was used for the light chain frameworks.
20   Several criteria were used to select murine
     framework residues in the design of the TF8-5G9 CDR-
     grafted heavy and light chain variable regions.
     Framework residues which, at a particular position, are
     idiosyncratic to TF8-5G9 were retained as murine
25   sequence with the assumption that they contributed to
     its unique binding characteristics. TF8-5G9 murine
     residues were also retained at framework positions where
     they were in agreement with the human consensus sequence
     but where the corresponding residues in KOL or REI were
30   idiosyncratic. Residues that are part of antibody loop
     canonical structures such as residue 71 (numbering

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according to Kabat et al.) of the heavy and light chains
1 were also retained as murine sequence. Framework
residues that form loops such as residues 26-30 of the
HC were kept as TF8-5G9 murine sequence at positions
were the murine sequence differed from the human.
5 Residues known to directly influence the conformation of
CDRs, such as 48 and 49 immediately preceding CDR2 of
the HC, were also retained as murine sequence.

The amino acid sequence of the variable region
for the initially designed CDR-grafted TF8-5G9 HC,
10 TF8HCDR1, is shown in SEQ ID NO:11. Murine residues
were retained at framework positions 6, 17, 23, 24, 28,
29, 30, 48, 49, 68, 71, 73, 78 88 and 91. The CDR-
grafted HC variable region was attached to a human IgG4
constant region.
15   The amino acid sequence of the variable region
for the initially designed CDR-grafted TF8-5G9 LC,
TF8LCDR1, is shown in SEQ ID NO:12. Murine residues
were retained at framework positions 39, 41, 46 and 105.
The CDR-grafted LC variable region was attached to a
20 human kappa constant region.

The variable region for the CDR-grafted HC and
LC described above were each assembled from 13 synthetic
oligonucleotides which were synthesized by Research
Genetics, Inc., Huntsville, AL. These oligonucleotides
25 ranged in length from 42 to 80 bases, and encoded both
variable region strands. When the 6 complementary
oligonucleotide pairs were annealed, the overhangs
generated were 17 to 24 bases in length. These
oligonucleotide pairs were combined, annealed at their
30 complementary overhangs, and ligated to give the final
full length double-stranded variable regions.

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The HC variable region oligonucleotides were
1 assembled into a 452 bp fragment which contains a 5'
EcoRI site and a 3' SacI site. The polymerase chain
reaction was used to amplify this fragment. The
resulting amplified DNA was purified on a 2% Nusieve, 1%
5 Seakem agarose gel (FMC). The appropriate size band of
DNA was excised and the DNA was recovered by the
Geneclean (Bio 101) procedure. The fragment was then
digested with EcoRI and SacI, and purified again by the
Geneclean method. This HC variable region fragment with
10 EcoRI and SacI ends was cloned into the EcoRI and SacI
sites of the pSport-1 vector (GIBCO-BRL Life
Technologies, Gaithersburg, MD). DNA from several
clones was isolated and sequenced to verify proper
variable region assembly. All clones had unexpected
15 base changes. One clone with the fewest base changes
(two mismatches at bases 133 and 140) was selected to be
corrected by site-directed mutagenesis according to
Kunkel (1985) Proc. Natl. Acad. Sci. USA 82:488.
Briefly, CJ236 (ung-, dut-) competent cells (Invitrogen
20 Corporation, San Diego, CA) were transformed with the
pSport vector containing the CDR-grafted HC variable
region with the two base mismatch. Single-stranded,
uridine-incorporated DNA templates were purified from
phage following M13 helper phage (Stratagene Cloning
25 Systems) infection of the transformed cells.
Mutagenesis oligos containing the desired base changes
were synthesized on an Applied Biosystems Model 380B DNA
synthesizer. The mutagenesis oligos were annealed to
the template DNA, and T7 DNA Polymerase and T4 DNA
30 Ligase (MutaGene InVitro Mutagenesis Kit, Bo-Rad
Laboratories, Richmond, CA) were used to incorporate the

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oligo into a newly synthesized DNA strand. DH5α
1 competent cells (GIBCO-BRL Life Technologies) were
transformed with the double-stranded DNA. The original
uridine-incorporated strand is destroyed while the newly
synthesized strand containing the mutagenesis oligo is
5 replicated. Phagemid DNA was prepared from the
resulting mutagenesis clones and the variable regions
were sequence to identify the clones which had
incorporated the desired changes. The corrected HC
EcoRI/SacI variable region fragment was excised from the
10 pSport vector, purified and ligated into the EcoRI/SacI
sites of a pSG5 vector containing the human IgG4
constant region. This resulted in the generation of a
full-length humanized TF8-5G9 HC gene, TF8HCDR1, in the
pSG5 COS cell expression vector. The vector was
15 designated pSG5TF8HCDR1.

The CDR-grafted TF8-5G9 LC variable region was
also amplified by the PCR from the assembled synthetic
oligonucleotides into a 433 bp fragment which contained
a 5' EcoRI site and a 3' NarI site. This fragment was
20 purified as described above for the HC, digested with
EcoRI and NarI and purified by the Geneclean procedure.
This fragment was cloned into the EcoRI and NarI sites
of a pSG5 vector which contains the human kappa constant
region. This resulted in the generation of a full-
25 length humanized TF8-5G9 LC gene, TF8LCDR1, in the pSG5
COS cell expression vector. Seven clones were
sequenced, and one was found to have the desired CDR-
grafted LC sequence. The vector was designated
pSQ5TF8LCDR1.
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EXAMPLE 4

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Expression of the CDR-Grafted
Heavy and Light Chain Genes in COS Cells

The transient expression of antibody genes in
5 COS-1 cells provides a rapid and convenient system to
test antibody gene expression and function. COS-1 cells
were obtained from the American Type Culture Collection
(CRL 1650) and cultured in Dulbecco's Modified Eagle
Medium (DMEM, from GIBCO BRL Life Technologies) with 10%
10 fetal calf serum. The pSG5TF8HCDR1 expression factor
was cotransfected into COS cells with the pSG5 chimeric
LC expression vector using the DEAE-Dextran method
followed by DMSO shock as described by Lopata et al.
(1984) Nucleic Acids Res. 14:5707. After 4 days of
15 culture, media was harvested from the wells and examined
for antibody expression levels.

Antibody levels were determined by an ELISA-
based assembly assay. Plates were coated with a goat
anti-human Fc specific antibody. Various dilutions of
20 the COS cell supernatant containing secreted antibody
were added, incubated for one hour, and washed. A
horseradish peroxidase-linked goat anti-human kappa
chain antibody was added, incubated for one hour at room
temperature, and washed. Substrate for the horseradish
25 peroxidase was added for detection. Antibody levels in
the COS cell media were found to be nearly undetectable
for the TF8HCDR1 x chimeric LC. Upon closer examination
of the TF8HCDR1 variable region sequence, it was found
that an unexpected base change, which had occurred
30 during the site-directed mutagenesis process described
in Example 3, introduced a stop codon into framework 4

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of the TF8HCDR1 gene. This substitution was corrected
1 by site-directed mutagenesis as described above.
Thorough sequencing of the variable region confirmed
that the correction was made with no additional changes
introduced. Upon transfection of this corrected
5 TF8HCDR1 gene with the chimeric LC, reasonable
expression levels were obtained.

      COS cells which had been co-transfected with
the CDR-grafted LC expression vector, pSGTF8LCDR1, and
either the chimeric HC or TF8HCDR1, produced antibody at
10 reasonable levels. Antibody levels in COS cell
supernatants ranged from 0.5 μg to 10.0 μg per ml.

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EXAMPLE 5

1   Binding of the CDR-Grafted TF8-5G9 to Tissue Factor

An ELISA was used to determine the ability of
the CDR-grafted TF8-5G9 antibody, TF8HCDR1 x TF8LCDR1,
5 to bind to tissue factor. Tissue factor was immobilized
on a -microtiter plate. The test COS cell supernatant,
containing the CDR-grafted antibody, was added to the
well, incubated for one hour at room temperature.
Following three washes with PBS/Tween, a goat anti-human
10 kappa chain polyclonal antibody conjugated to
horseradish peroxidase was added, incubated for one hour
at room temperature and washed. Substrate for the
horseradish peroxidase was added for detection. The
positive control was the TF8-5G9 chimeric antibody. The
15 CDR-grafted TF8-5G9 antibody was able to bind to tissue
factor to a degree comparable to the chimeric TF8-5G9
antibody (Figure 3, solid symbols).
The ability of the humanized antibody to
compete with murine TF8-5G9 for binding to tissue factor
20 was also examined. Varying amounts of COS cell
supernatant containing the test CDR-grafted antibody and
a fixed amount of murine TF8-5G9 were added
simultaneously to wells coated with tissue factor.
Binding was allowed to occur for one hour at room
25 temperature. The wells were washed three times with
PBS/Tween. A goat anti-human kappa chain antibody
conjugated to horseradish peroxidase was added,
incubated for one hour at room temperature and washed.
Substrate for the horseradish peroxidase was added for
30 detection. The positive antibody competed as well as

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the chimeric antibody with murine TF8-5G9 for binding to

1 TF.

            These data indicate that the initially
designed CDR-grafted antibody, TF8HCDR1 x TF8LCDR1, was
approximately as active as the chimeric TF8-5G9 in

5 binding to TF and competing with the murine antibody for
binding to TF.

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EXAMPLE 6

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Construction and Characterization
of Additional CDR-Grafted Heavy Chains

Upon examination of the molecular structure of

5 murine TF8-5G9, framework residues at positions 27, 68,
73 and 78 were found to lie on the antibody surface and
had no discernible contact with the CDRs. These
framework residues were of murine sequence in TF8HCDR1
but were changed to the human KOL sequence in various

10 combinations to generate a series of CDR-grafted heavy
chains with framework residue variations. The changes
were made by the process of site-directed mutagenesis as
described in Example 3. Each CDR-grafted heavy chain
version was expressed in COS cells in combination with

15 the CDR-grafted LC, TF8LCDR1, and tested for its ability
to bind TF and compete with murine TF8-5G9 for binding.
Every version of the CDR-grafted heavy chain in
combination with TF8LCDR1 was shown to bind TF with an
affinity comparable to chimeric TF8-5G9. Every CDR-

20 grafted HC in combination with TF8LCDR1 was able to
compete with murine TF8-5G9 for binding to TF to a
degree comparable to the chimeric antibody.

Changes in sequence from murine to human for
HC framework positions 6, 7, 68, 73 and 78 did not

25 adversely affect the antigen binding ability of the
antibody. The CDR-grafted HC version which had human
sequence at all of these positions, and thus was the
most humanized HC, was TF8HCDR20.

The complete sequence of the TF8HCDR20 gene

30 was determined. The DNA sequence is shown as a 2360 bp
EcoRI/BamHI insert with protein translation in the

35

pEe6TF8HCDR20 expression vector in Figure 4 and SEQ ID
1 NO:15.

The essential regions of the gene are as
follows:

Nucleotide #	Region
1-6	5' EcoRI restriction site
- 7-15	Kozak sequence
16-72	Start codon and leader sequence
73-423	CDR-grafted variable region
424-717	Human IgG4 CH1 domain
718-1110	Human IgG4 intron 2
1111-1146	Human IgG4 hinge
1147-1267	Human IgG4 intron 3
1268-1594	Human IgG4 CH2 domain
1595-1691	Human IgG4 intron 4
1692-2012	Human IgG4 CH3 domain
2013-2354	3' untranslated region
2355-2360	3' BamHI end spliced to BclI site of the expression vector

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EXAMPLE 7

1

Construction and Characterization
of Additional CDR-Grafted Light Chains

The initially designed CDR-grafted LC,

5 TF8LCDR1, contained four framework residues from the
murine TF8-5G9 sequence. At two of these positions, 39
and 105, the human REI framework sequence is unique to
REI; however, the murine TF8-5G9 LC sequence is in
agreement with the human consensus sequence. The other

10 two murine framework residues, trp41 and thr46, are
unique to TF8-5G9. Several versions of the CDR-grafted
LC were generated in which the sequence at these four
positions were changed from the murine to the human REI
in various combinations. These changes were made by

15 site-directed mutagenesis. Each version of the CDR-
grafted LC was expressed in COS cells in combination
with the CDR-grafted HC, TF8HCDR20, and tested for
ability to bind tissue factor and compete with murine
TF8-5G9 for binding. Every version of the CDR-grafted

20 LC, in combination with TF8HCDR20, was shown to bind TF
with an affinity comparable to TF8-5G9. Also every CDR-
grafted LC version, in combination with TF8HCDR20, was
able to compete with murine TF8-5G9 for binding to TF in
a manner comparable to the chimeric TF8-5G9 control.

25   Changes in sequence from murine to human for
LC framework positions 39, 41, 46 and 105 did not
adversely effect the ability of the antibody to
recognize antigen. The CDR-grafted LC of choice was
TF8LCDR3, where murine TF8-5G9 sequence was used at

30 positions 39 and 105 because these are in agreement with

35

1 the human consensus sequence. The preferred CDR-grafted
TF8-5G9 antibody is TF8HCDR20 x TF8LCDR3.

The complete sequence of the TF8LCDR3 gene was
determined and is shown as a 759 bp EcoRI-BamHI insert
with protein translation in the pEe12TF8LCDR3 expression
5 vector in Figure 5 and SEQ ID NO:17. The essential
regions of the gene are as follows:

Nucleotide #	Region
1-5	5' EcoRI restriction site
6-8	Kozak sequence
9-68	Start codon and leader sequence
69-392	CDR-grafted variable region
393-710	Human kappa constant region
711-753	3' untranslated region
754-759	3'BamHI end spliced to BclI site of the expression vector

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EXAMPLE 8

1   CDR-Grafted TF8-5G9 Antibody TF8HCDR20 x TF8LCDR3
                    Inhibits Human Tissue Factor

                The binding of the CDR-grafted TF8-5G9
5   antibody, TF8HCDR20 x TF8LCDR3, to TF was assessed as
     described in Example 5 and was found to be comparable to
     that of the chimeric TF8-5G9 as illustrated in Figure 6.
     The ability of the CDR-grafted TF8-5G9 to compete with
     the murine antibody for binding to TF is comparable to
10   that of the chimeric TF8-5G9 as shown in Figure 7.
                An _in_ _vitro_ assay was used to measure the
     level of inhibition of factor X activation by the CDR-
     grafted TF8-5G9 antibody. In this assay, TF forms an
     active proteolytic complex with factor VII. This
15   complex then converts factor X to factor Xa by
     proteolysis. The activated Xa enzymatically cleaves a
     substrate, Spectrozyme FXa, which releases a chromogen.
     The level of chromogen, as detected by optical density,
     is an indication of factor X activation due to TF-factor
20   VIIa activity.
                The following reaction mixtures were prepared
     in 12 x 75 mm borosilicate glass tubes.
                    25 μl TBS (50 mM Tris, pH 7.4, 150 mM NaCl)
                    15 μl 20 mM $CaCl_2$/1% bovine serum albumin
25   (BSA)
                    20 μl human placental tissue factor solution
                        (prepared by reconstituting one vial of
                        Thromborel S, Curtin Matheson Scientific
                        #269-338 with 4.0 ml dH_2O and diluting
30   1:10 in TBS)

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1   30 μl Factor VII (Enzyme Research Labs #HFVII
               1007 at 237.66 ng/ml in TBS)
         30 μl TBS or TF8-5G9 or TF8MCDR20 x TF8LCDR3
               at 1.18 μg/ml or as indicated in Fig. 8
         The reaction mixtures were incubated at 37°C
5   for ten minutes before the addition of Factor X. (In
    some cases the reaction mixture was preincubated for
    five minutes before addition of Factor VII or antibody,
    followed by a ten minute incubation before addition of
    Factor X.) Thirty μl of Factor X solution (Enzyme
10 Research Labs, DHFX 330, 247.38 μg/ml TBS) was added and
    the mixture was incubated at 37°C for three minutes.
    Factor X activation was terminated by pipetting 40 μg of
    reaction mixture into 160 μl of stop buffer (50 mM Tris,
    pH 7.4, 100 mM EDTA, 150 mM NaCl) in 96 well microtiter
15 plates. Each tube of reaction mixture was pipetted into
    three microtiter wells. Fifty μl of Spectrozyme FXa
    substrate (American Diagnostica #222, 1μM/ml TBS) was
    added to each well. OD_{405} was read on a Molecular
    Devices kinetic plate reader with readings taken every
20 twenty seconds for ten minutes. Factor X activity was
    recorded as mOD/minute, and enzyme velocities over the
    linear portion of the reaction curve were compared to
    determine inhibition of factor X activation by the anti-
    TF antibodies.
25   As shown in Figure 8, the CDR-grafted TF8-5G9
    antibody is approximately as effective as the murine
    TF8-5G9 in inhibiting factor X activation. This
    indicates that the CDR-grafted TF8-5G9 is functionally
    active.
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EXAMPLE 9

1   Construction of the CDR-Grafted Heavy
                and Light Chain Myeloma Expression Vectors

            For the purpose of establishing a permanent
5   CDR-grafted antibody-producing cell line, the TF8HCDR20
    and TF8LCDR3 genes were subcloned into myeloma cell
    expression vectors. The heavy chain TF8HCDR20 was
    subcloned into the EcoRI and BclI sites of the pEe6hCMV-
    BglII myeloma expression vector described by Stephens et
10 al. (1989) Nucleic Acids Res. 17:7110 to produce
    pEe6TF8HCDR20. The light chain TF8LCDR3 was subcloned
    into the EcoTI and BclI sites of the pEe12 myeloma
    expression vector to produce pEe12TF8LCDR3. The heavy
    and light chain expression vectors are illustrated in
15 Figures 9 and 10, respectively. In both vectors
    antibody gene transcription was driven by the human
    cytomegalovirus (hCMV) promoter-enhancer, which lies
    directly 5' to the multiple cloning site. The
    polyadenylation signal sequence lies 3' to the multiple
20 cloning site and signals the termination of
    transcription. Each vector contains the ß-lactamase
    gene to allow for ampicillin selection in E. coli. The
    pEe12 vector contains a glutamine synthetase cDNA gene
    under the transcriptional control of the SV40 early
25 promoter. Glutamine synthetase allows for myeloma cell
    transfectants to be selected in glutamine-free media.
    Myeloma cells are devoid of glutamine synthetase
    activity and are dependent on a supply of glutamine in
    the culture media. Cells which have been transfected
30 with the pEe12 vector, containing the glutamine

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synthetase gene, are able to synthesize glutamine from
1 glutamate and can survive in the absence of glutamine.

The pEe6TF8HCDR20 expression vector is a 7073 bp
plasmid whose DNA sequence is shown in Figure 4 and SEQ
ID NO:15. The coding regions of the TF8HCDR20 gene are
5 translated. The essential regions of this vector are
described below:

> 1. Nucleotides #1-2360: The TF8HCDR20 CDR-
>   grafted HC gene is described in Example
>   6. The HC gene was inserted as an
>   EcoRI/BamHI fragment into the EcoRI/BclI
10   sites of the pEe6hCMV-BglII vector.
>
> 2. Nucleotides #2361-2593: This region
>   encodes the SV40 early gene
>   polyadenylation signal (SV40 nucleotides
>   2770-2537), which acts as a
>   transcriptional terminator. This
>   fragment is flanked by a 5' BclI site and
15   a 3' BamHI site. The 3' BamHI end of the
>   heavy chain gene was spliced to the 5'
>   BclI site of the polyadenylation signal,
>   thus eliminating both sites.
>
> 3. Nucleotides #2594-3848: This region is a
>   BamHI-BglI fragment from pBR328
20   (nucleotides 375-2422) but with a
>   deletion between the SaI and AvaI sites
>   (pBR328 nucleotides 651-1425) following
>   the addition of a SalI linker to the AvaI
>   site. This region contains the Col El
>   bacterial origin of replication.
>
> 4. Nucleotides #3849-4327: This is a BglI-
25   XmnI fragment site from the ß-lactamase
>   gene of pSP64 (Promega Corporation,
>   Madison, WI). This gene provides
>   ampicillin resistance to bacteria
>   transformed with this vector.
>
> 5. Nucleotides #4328-4885: This is an XmnI-
30   HindIII fragment of the ColEl based
>   plasmid pCT54 described by Emtage et al.
>   (1983) Proc. Natl. Acad. Sci. USA

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<u>80</u>:3671. The <u>Hind</u>III site was converted
to a <u>Bgl</u>II site by the addition of a
linker following the addition of the hCMV
promoter described below.

6. Nucleotides #4886-7022: These
    nucleotides encode the <u>Pst</u>-1m fragment of
    human cytomeglovirus (h<u>CMV</u>) strain AD 169
    described by Greenway <u>et</u> <u>al</u>. (1982) <u>Gene</u>
    <u>18</u>:355 containing the region coding for
    the hCMV middle intermediate early
    promoter. This <u>Pst</u>-1m fragment was
    cloned into the <u>Hind</u>III site of pEe6hCMV
    by addition of oligonucleotides of the
    following sequence to either end of the
    fragment:

    5' GTCACCGTCCTTGACACGA 3'

    3' ACGTCAGTGGCAGGAACTGTGCTTCGA 5'

    The resulting 2100 bp fragment was
    inserted such that the promoter directed
    transcription towards the <u>Eco</u>RI site of
    pEe6hCMV. The oligonucleotide above
    served to recreate the complete 5'
    untranslated sequence of the hCMV-MIE
    gene the added irrelevant sequence at the
    very 5' end of the fragment. The <u>Hind</u>III
    site at the 5' end was subsequently
    converted to a <u>Bgl</u>II site by the addition
    of a further linker.

7. Nucleotides #7023-7073: The pSP64
    polylinker with the <u>Bam</u>HI and <u>Sal</u>I sites
    removed.

The pEe12TF8LCDR3 expression vector is a 7864
bp plasmid whose DNA sequence is shown in Figure 5 and
SEQ ID NO:17. The coding regions of the TF8LCDR3 gene
are translated. The essential regions of this
expression vector are described below:

1. Nucleotides #1-759: The TF8LCDR3 CDR-
    grafted LC gene is described in Example
    7. The gene was inserted as an

1   EcoRI/BamHI fragment into the EcoRI/BclII
        sites of the pEe12 expression vector.

   2.   Nucleotides #760-3284: These regions of
        pEe12 are identical to the regions
        encoded by nucleotides 2361-4885 of the
        pEe6TF8HCDR20 vector described above
5   (regions #2-5).

   3.   Nucleotides #3285-5736: This region
        encodes the Chinese hamster ovary
        glutamine synthetase cDNA under the
        transcriptional control of the SV40 early
        promoter and followed by the SV40
        polyadenylation and splice signals from
10   the pSV2.dhfr vector described by
        Subramani et al. (1981) Mol. Cell. Biol.
        1:854. The following describes the
        derivation of this region: A 1200 bp
        NaeI-PvuII fragment, containing a
        complete GS coding sequence, was excised
        from the Chinese hamster ovary cDNA clone
        λGS1.1 described by Hayward et al. (1986)
15   Nucleic Acid Res. 14:999. After addition
        of a HindIII linker to the NaeI site and
        a BglII linker to the PvuII site (hence
        destroying the NaeI and PvuII sites), the
        1200 bp fragment was cloned in place of
        DHFR sequences in pSV2.dhfr between the
        HindIII and BglII sites to form pSV2.GS.
20   The single remaining PvuII site in
        pSV2BamGS was converted to a BamHI site
        by addition of an oligonucleotide linker
        to form pSV2BamGS. An EcoRI site in the
        GS cDNA was destroyed by site directed
        mutagenesis without altering the amino
        acid sequence in pSV2BamGS and the
25   HindIII site was destroyed by filling in
        with DNa polymerase I. The 2451 bp BamHI
        fragment from this plasmid, containing
        the complete SV40-GS hybrid transcription
        unit, was excised and inserted at the
        BglII site of pEe6hCMV-BglII site of
        pEe6hCMV-BglII such that transcription
        from the sV40 early promoter proceeds
30   towards the hCMV promoter.

35

4.   Nucleotides #5737-7864: This region is
1   identical to the hCMV promoter and pSP64
           polylinker encoded by nucleotides 4886-
           7073 of the pEe6TF8HCDR20 vector
           described above (regions 6 and 7).

        For the purpose of ensuring that both the
5   pEe6TF8HCDR20 and peE12TF8LCDR3 vectors co-transfected
    myeloma cells, the vectors were joined in linear
    concatamers. Both the pEe6TF8HCDR20 and pEe12TF8LCDR3
    vectors were digested at the unique SalI site. The SalI
    linearized pEe6TF8HCDR20 vector was phosphatased at its
10 5' ends to prohibit ligation of two pEe6TF8HCDR20
    vectors onto each other. This phosphatased HC vector
    was ligated in a 2:1 molar ratio to the Sal linearized
    pEe12TF8LCDR3. The resulting concatamers were most
    likely of the following composition:

15   SalI   SalI   SalI   SalI
    ┌──────────────────┬──────────────────┬──────────────────┐
    │ pEe6TF8HCDR20   │ pEe12TF8LCDR3   │ pEe6TF8HCDR20   │
    └──────────────────┴──────────────────┴──────────────────┘
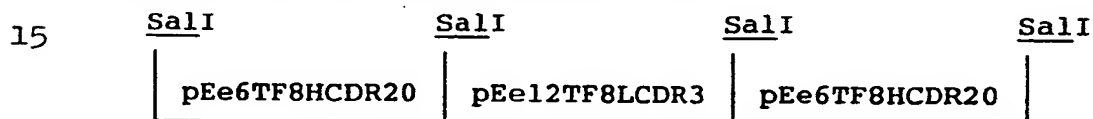
    This concatamerized DNA was extracted with phenol and
    chloroform, and precipitated with ammonium acetate and
20 ethanol. The DNA precipitate was resuspended in
    distilled water to a concentration of 1 μg/μL and used
    to transfect myeloma cells.

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EXAMPLE 10

1   Development of NSO Expression Cell Lines

          Stably transformed cell lines expressing the
humanized TF8-5G9 antibody were prepared by transfecting
5 CDR-grafted heavy and light chain expression vectors
into NSO mouse myeloma cells. Selection of transfected
cells was carried out using the dominant selectable
marker gene, glutamine synthetase (GS).

          The NSO mouse myeloma cell line, obtained from
10 Celltech, Ltd., is a subclone derived from NS-1 and does
not express intracellular light chains.. These cells
were cultured in Dulbecco's modified Eagle's medium
(DMEM) with added glutamine and 10% fetal bovine serum
(FBS). To prepare for transfection, the cells were
15 harvested in mid-log phase of the growth cycle,
centrifuged for 5 minutes, washed with phosphate
buffered saline (PBS), centrifuged again, and the cell
pellet was resuspended in 2.2 mL of PBS. The final cell
concentration was 2.18×10^7 mL. Cells were maintained
20 on ice during the entire procedure.

          The DNA to be transfected (pEe12TF8LCDR3 x
pEe6TF8HCDR20) was prepared as a concatamer as described
in Example 9. The DNA and NSO cells were added to a 0.4
cm BioRad Gene Pulser cuvette in the following order:
25   40 μL (40 μg) DNA concatamer
             320 μL double distilled water
             40 μL 10 x PBS
             400 μL NSO cells (8.72×10^6 cells)
          Transfection was performed by electroporation
30 following a protocol provided by Celltech, Ltd. In this
procedure, the cells and DNA in PBS buffer were exposed

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to a brief, high voltage pulse of electricity causing
1 transient micropores to form on the cell membrane. DNA
   transfer takes place through these openings. To prepare
   for electroporation, the suspension of NSO cells and DNA
   was gently mixed and incubated on ice for 5 minutes.
5 The cuvette was placed in a BioRad Gene Pulser and given
   2 consecutive electrical pulses at settings of 3 μF
   (capacitance) and 1.5V (voltage). Following
   electroporation, the cuvette was returned to the ice for
   5 minutes. The suspension was then diluted in prewarmed
10 growth medium and distributed into seven 96-well plates.
   Control plates containing cells electroporated without
   DNA were also prepared at the same time to measure the
   presence of spontaneous mutants. Plates were placed in
   a 37°C incubator with 5% CO_2.
15   Glutamine synthetase, encoded by the GS gene,
   is an enzyme that converts glutamate to glutamine. NSO
   cells require glutamine for growth due to inadequate
   levels of endogenous GS gene expression. In the DNA
   concatamer, this gene is located on the pEe12TF8LCDR3
20 vector. Transfected cells which incorporate the GS gene
   become glutamine-independent. Cells not integrating the
   GS gene into their genome would remain glutamine-
   dependent and would not survive in glutamine-free
   medium. Approximately 18 hours post electroporation,
25 all plates were fed with glutamine-free selection medium
   and returned to the incubator until viable colonies
   appeared.
            Approximately 3 weeks after transfection,
   distinct macroscopic colonies were observed. These were
30 screened for expression of the intact humanized antibody
   using the assembly ELISA as described in Example 5.

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Tissue culture supernatants from wells containing

1 colonies were screened at a 1:10 dilution. Positive
wells showing activity greater than the 25 ng/mL
standard were subcultured and expanded for further
analysis.

5   For selection of high producers, antibody
production was quantitated after a 96 hour growth
period. Tissue culture flasks were seeded with 2 x 10^5
cells/mL in 10 mL of selection medium and incubated at
37°C, 5% CO_2 for 96 hours. At the end of that time

10 period, an aliquot was taken to determine cell
concentration and antibody titer. Evaluation of
antibody production was calculated as μg/mL and
pg/cell/96 hours. The highest producers from this
transfection were:

15   **Cell Line**   **μg/mL**   **pg/cell/96 hour**
            2B1   26.3   24.3
            3E11   27.6   59.9
            4G6   30.2   41.9

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EXAMPLE 11
CDR Grafted Antibody TF8HCDR20 x TF8LCDR3
Inhibits Tissue Factor In Vivo

CDR grafted antibody TF8HCDR20 x TF8LCDR3 was
compared to murine antibody TF8-5G9 for its ability to
protect rats from experimentally induced disseminated
intravascular coagulation (DIC). In the DIC model, rats
are challenged with human thromboplastin (a crude tissue
extract containing TF activity), resulting in fibrinogen
consumption and death. Pretreatment of rats with anti-
TF antibody was demonstrated to protect rats from
fibrinogen consumption and death as follows.

Human thromboplastin was prepared as described
in U.S. Patent 5,223,427. Saline control or 30 μ/ml of
TF8-5G9 or CDR-grafted antibody was injected through the
tail vein of rats, followed by injection of
thromboplastin equivalent to 200 ng of recombinant TF.
Clotting times were determined at T=0 and T=1 minute as
a measure of fibrinogen concentration. Clotting times
are proportional to fibrinogen concentration, with a 60
second clotting time corresponding to an 80% reduction
in fibrinogen concentration. Clotting times of greater
than 60 seconds cannot be accurately measured and were
recorded as 60 seconds.

Survivability and clotting times for three
representative studies are shown below.

Survivors

Study	Controls	TF8-5G9	CDR-grafted Ab
1	0/8	5/8	6/8
2	0/8	4/7	7/8
3	0/8	8/8	3/7

Clotting Times
Controls

	Study #1			Study #2			Study #3	
	T=0	T=1		T=0	T=1		T=0	T=1
	16	>60		18	>60		19	>60
	16	>60		18	>60		21	>60
	16	>60		18	>60		18	>60
	17	>60		18	>60		19	>60
	15	>60		16	>60		18	54
	16	>60		18	>60		18	>60
	16	>60		17	>60		18	>60
	16	>60		17	>60		18	>60

Clotting Times
Murine TF8-5G9

	Study #1			Study #2			Study #3	
	T=0	T=1		T=0	T=1		T=0	T=1
	16	36		18	34		19	28
	15	41		18	36		18	29
	15	33		18	>60		19	29
	15	31		17	>60		18	29
	15	>60		18	50		18	28
	16	>60		17	34		19	40
	16	33		17	34		19	40
	16	33		18	31		19	34
	16	>60					19	>60

Clotting Times
CDR-grafted TF8-5G9

	Study #1			Study #2			Study #3	
	T=0	T=1		T=0	T=1		T=0	T=1
	16	>60		17	>60		21	>60
	16	>60		17	33		18	34
	16	>60		18	32		17	>60
	22	37		18	>60		20	35
	16	32		17	32		17	58
	15	>60		18	31		18	33
	16	>60		17	31		18	31
	16	>60		16	32			

Twenty-three of the twenty-four control rats

1   had clotting times of greater than 60 seconds indicating
     that virtually all untreated rats were consuming more
     than 80% of their fibrinogen. Both the CDR-grafted and
     murine antibody treated rats had similar clotting times

5   at one minute of 44.5 and 40 seconds. Further, only six
     of the murine antibody treated rats and nine of the CDR-
     grafted antibody treated rats had clotting times in
     excess of 60 seconds. Accordingly, both the murine and
     CDR-grafted antibodies were able to neutralize TF and

10   thus protect rats from fibrinogen consumption and death.

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SEQUENCE LISTING

1

(1) GENERAL INFORMATION:

    (i) APPLICANT: Joliffe, Linda K.
                Zivin, Robert A.

5   Pulito, Virginia L.

    (ii) TITLE OF INVENTION: CDR-GRAFTED ANTI-TISSUE FACTOR
        ANTIBODIES AND METHODS OF USE THEREOF

    (iii) NUMBER OF SEQUENCES: 20

    (iv) CORRESPONDENCE ADDRESS:
        (A) ADDRESSEE: Scully, Scott, Murphy & Presser
10   (B) STREET: 400 Garden City Plaza
        (C) CITY: Garden City
        (D) STATE: New York
        (E) COUNTRY: United States
        (F) ZIP: 11530

    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
15   (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

    (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER:
        (B) FILING DATE: 07-JUN-1995
        (C) CLASSIFICATION:

   (viii)-ATTORNEY/AGENT INFORMATION:
        (A) NAME: DiGiglio, Frank S.
20   (B) REGISTRATION NUMBER: 31,346
        (C) REFERENCE/DOCKET NUMBER: 9598

    (ix) TELECOMMUNICATION INFORMATION:
        (A) TELEPHONE: (516) 742-4343
        (B) TELEFAX: (516) 742-4366
        (C) TELEX: 230 901 SANS UR

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(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1489 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 11..1391

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GGTCCTTACA ATG AAA TGC AGC TGG GTC ATC TTC TTC CTG ATG GCA GTG        49
           Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val
            1               5                   10

GTT ACA GGG GTC AAT TCA GAG ATT CAG CTG CAG CAG TCT GGG GCT GAG        97
Val Thr Gly Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu
        15                  20                  25

CTT GTG AGG CCA GGG GCC TTA GTC AAG TTG TCC TGC AAA GCT TCT GGC       145
Leu Val Arg Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly
 30                  35                  40                  45

TTC AAC ATT AAA GAC TAC TAT ATG CAC TGG GTG AAG CAG AGG CCT GAA       193
Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu
                50                  55                  60

CAG GGC CTG GAG TGG ATT GGA TTG ATT GAT CCT GAG AAT GGT AAT ACT       241
Gln Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr
                65                  70                  75

ATA TAT GAC CCG AAG TTC CAG GGC AAG GCC AGT ATA ACA GCA GAC ACA       289
Ile Tyr Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr
                80                  85                  90

TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT GAG GAC       337
Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp
        95                  100                 105

ACT GCC GTC TAT TAC TGT GCT AGA GAT AAC TCG TAC TAC TTT GAC TAC       385
Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr
110                 115                 120                 125
```

```
    TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC AAA ACG ACA CCC          433
  1 Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro
                      130             135             140

    CCA TCT GTC TAT CCA CTG GCC CCT GGA TCT GCT GCC CAA ACT AAC TCC          481
    Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser
                145             150             155

    ATG GTG ACC CTG GGA TGC CTG GTC AAG GGC TAT TTC CCT GAG CCA GTG          529
  5 Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val
                160             165             170

    ACA GTG ACC TGG AAC TCT GGA TCC CTG TCC AGC GGT GTG CAC ACC TTC          577
    Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe
                175             180             185

    CCA GCT GTC CTG CAG TCT GAC CTC TAC ACT CTG AGC AGC TCA GTG ACT          625
 10 Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr
    190             195             200             205

    GTG CCC TCC AGC ACC TGG CCC AGC GAG ACC GTC ACC TGC AAC GTT GCC          673
    Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala
                    210             215             220

    CAC CCG GCC AGC AGC ACC AAG GTG GAC AAG AAA ATT GTG CCC AGG GAT          721
    His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp
                225             230             235
 15
    TGT GGT TGT AAG CCT TGC ATA TGT ACA GTC CCA GAA GTA TCA TCT GTC          769
    Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val
                240             245             250

    TTC ATC TTC CCC CCA AAG CCC AAG GAT GTG CTC ACC ATT ACT CTG ACT          817
    Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr
                255             260             265

 20 CCT AAG GTC ACG TGT GTT GTG GTA GAC ATC AGC AAG GAT GAT CCC GAG          865
    Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu
    270             275             280             285

    GTC CAG TTC AGC TGG TTT GTA GAT GAT GTG GAG GTG CAC ACA GCT CAG          913
    Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln
                290             295             300

    ACG CAA CCC CGG GAG GAG CAG TTC AAC AGC ACT TTC CGC TCA GTC AGT          961
 25 Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser
                305             310             315
```

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```
      GAA CTT CCC ATC ATG CAC CAG GAC TGG CTC AAT GGC AAG GAG TTC AAA        1009
      Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys
  1       320             325             330

      TGC AGG GTC AAC AGT GCA GCT TTC CCT GCC CCC ATC GAG AAA ACC ATC        1057
      Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile
          335             340             345

      TCC AAA ACC AAA GGC AGA CCG AAG GCT CCA CAG GTG TAC ACC ATT CCA        1105
  5   Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro
      350             355             360             365

      CCT CCC AAG GAG CAG ATG GCC AAG GAT AAA GTC AGT CTG AAC TGC ATG        1153
      Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Asn Cys Met
                      370             375             380

      ATA ACA GAC TTC TTC CCT GAA GAC ATT ACT GTG GAG TGG CAG TGG AAT        1201
      Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn
 10               385             390             395

      GGG CAG CCA GCG GAG AAC TAC AAG AAC ACT CAG CCC ATC ATG GAC ACA        1249
      Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr
                  400             405             410

      GAT GGC TCT TAC TTC GTC TAC AGC AAG CTC AAT GTG CAG AAG AGC AAC        1297
      Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn
          415             420             425
 15
      TGG GAG GCA GGA AAT ACT TTC ACC TGC TCT GTG TTA CAT GAG GGC CTG        1345
      Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu
      430             435             440             445

      CAC AAC CAC CAT ACT GAG AAG AGC CTC TCC CAC TCT CCT GGT AAA T          1391
      His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys
                  450             455             460

 20   GATCCCAGTG TCCTTGGAGC CCTCTGGTCC TACAGGACTC TGACACCTAC CTCCACCCCT      1451

      CCCTGTATAA ATAAAGCACC CAGCACTGCC TTGGACCC                             1489
```

      (2) INFORMATION FOR SEQ ID NO:2:

          (i) SEQUENCE CHARACTERISTICS:
 25   (A) LENGTH: 460 amino acids
              (B) TYPE: amino acid
              (D) TOPOLOGY: linear

 30

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(ii) MOLECULE TYPE: protein

1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
    Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val Val Thr Gly
     1               5                  10                  15

5   Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg
                 20                  25                  30

    Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile
             35                  40                  45

    Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
         50                  55                  60

10  Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp
     65                  70                  75                  80

    Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr Ser Ser Asn
                     85                  90                  95

    Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val
                 100                 105                 110

    Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln
15           115                 120                 125

    Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val
             130                 135                 140

    Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr
    145                 150                 155                 160

    Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr
20                   165                 170                 175

    Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val
                 180                 185                 190

    Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser
                 195                 200                 205

    Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala
25      210                 215                 220
```

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```
      Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys
      225             230             235             240
  1
      Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe
                  245             250             255

      Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val
                  260             265             270

  5   Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe
                  275             280             285

      Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro
          290             295             300

      Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro
      305             310             315             320

 10   Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val
                  325             330             335

      Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr
                  340             345             350

      Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys
                  355             360             365

 15   Glu Gln Met Ala Lys Asp Lys Val Ser Leu Asn Cys Met Ile Thr Asp
          370             375             380

      Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro
      385             390             395             400

      Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser
                  405             410             415

 20   Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala
                  420             425             430

      Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His
                  435             440             445

      His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys
          450             455             460
```

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(2) INFORMATION FOR SEQ ID NO:3:

1   (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 937 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: double
                (D) TOPOLOGY: linear

5   (ii) MOLECULE TYPE: peptide

          (ix) FEATURE:
                (A) NAME/KEY: CDS
                (B) LOCATION: 5..706

          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
       GGAC ATG CGG GCC CCT GCT CAG TTT TTT GGG ATC TTG TTG CTC TGG TTT        49
10          Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe
            1               5                   10                  15

       CCA GGT ATC AGA TGT GAC ATC AAG ATG ACC CAG TCT CCA TCC TCC ATG        97
       Pro Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met
                        20              25                  30

       TAT GCA TCG CTG GGA GAG AGA GTC ACT ATC ACT TGT AAG GCG AGT CAG       145
       Tyr Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln
15                   35                  40                  45

       GAC ATT AGA AAG TAT TTA AAC TGG TAC CAG CAG AAA CCA TGG AAA TCT     . 193
       Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser
                    50                  55                  60

       CCT AAG ACC CTG ATC TAT TAT GCA ACA AGC TTG GCA GAT GGG GTC CCA       241
       Pro Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro
                65                  70                  75
20
       TCA AGA TTC AGT GGC AGT GGA TCT GGG CAA GAT TAT TCT CTA ACC ATC       289
       Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile
       80                  85                  90                  95

       AGC AGC CTG GAG TCT GAC GAT ACA GCA ACT TAT TAC TGT CTA CAA CAT       337
       Ser Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His
                        100                 105                 110

25     GGT GAG AGC CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAC       385
       Gly Glu Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn
                        115                 120                 125
```

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```
       AGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA TCC AGT GAG       433
       Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
   1           130             135             140

       CAG TTA ACA TCT GGA GGT GCC TCA GTC GTG TGC TTC TTG AAC AAC TTC       481
       Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
               145             150             155

       TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT GAT GGC AGT GAA CGA       529
   5   Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
       160             165             170             175

       CAA AAT GGC GTC CTG AAC AGT TGG ACT GAT CAG GAC AGC AAA GAC AGC       577
       Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
               180             185             190

       ACC TAC AGC ATG AGC AGC ACC CTC ACG TTG ACC AAG GAC GAG TAT GAA       625
       Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
   10          195             200             205

       CGA CAT AAC AGC TAT ACC TGT GAG GCC ACT CAC AAG ACA TCA ACT TCA       673
       Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
               210             215             220

       CCC AAT GTC AAG AGC TTC AAC AAG AAT GAG TGT TAGAGACAAA GGTCCTGAGA     726
       Pro Asn Val Lys Ser Phe Asn Lys Asn Glu Cys
               225             230
   15
       CGCCACCACC AGCTCCCCAG CTCCATCCTA TCTTCCCTTC TAAGGTCTTG GAGGCTTCCC    786

       CACAAGCGAC CTACCACTGT TGCGGTGCTC CAAACCTCCT CCCCACCTCC TTCTCCTCCT    846

       CCTCCCTTTC CTTGGCTTTT ATCATGCTAA TATTTGCAGA AAATATTCAA TAAAGTGAGT    906

       CTTTGCACTT GAAAAAAAAA AAAAAAAAAA A                                   937
   20
       (2) INFORMATION FOR SEQ ID NO:4:

           (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 234 amino acids
               (B) TYPE: amino acid
               (D) TOPOLOGY: linear
   25      (ii) MOLECULE TYPE: protein
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
1  Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe Pro
   1               5                   10                  15
   Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr
                   20                  25                  30
5  Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp
               35                  40                  45
   Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser Pro
           50                  55                  60
   Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser
       65                  70                  75                  80
10 Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser
                   85                  90                  95
   Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His Gly
               100                 105                 110
   Glu Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn Arg
           115                 120                 125
   Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln
15     130                 135                 140
   Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr
   145                 150                 155                 160
   Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln
                   165                 170                 175
   Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr
20                 180                 185                 190
   Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg
               195                 200                 205
   His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
       210                 215                 220
   Asn Val Lys Ser Phe Asn Lys Asn Glu Cys
25 225                 230
```

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35

(2) INFORMATION FOR SEQ ID NO:5:

1   (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 5 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: peptide
5

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

      Asp Asp Tyr Met His
      1   5

10 (2) INFORMATION FOR SEQ ID NO:6:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 17 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: peptide
15

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

      Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Lys Pro Lys Phe Gln
      1   5   10   15

      Gly

20

(2) INFORMATION FOR SEQ ID NO:7:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 8 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear
25
       (ii) MOLECULE TYPE: peptide

30

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

1   Asp Asn Ser Tyr Tyr Phe Asp Tyr
       1   5

   (2) INFORMATION FOR SEQ ID NO:8:

5   (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 11 amino acids
           -   (B) TYPE: amino acid
               (C) STRANDEDNESS: double
               (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: peptide

10   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

       Lys Ala Ser Gln Asp Ile Arg Lys Tyr Leu Asn
       1   5   10

   (2) INFORMATION FOR SEQ ID NO:9:

15   (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 7 amino acids
               (B) TYPE: amino acid
               (C) STRANDEDNESS: double
               (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: peptide

20   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

       Tyr Ala Thr Ser Leu Ala Asp
       1   5

25

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(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 9 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

    Leu Gln His Gly Glu Ser Pro Tyr Thr
    1   5

(2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 117 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

    Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
    1   5   10   15

    Leu Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr
                20   25   30

    Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
                35   40   45

    Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp Pro Lys Phe
                50   55   60

    Gln Gly Arg Phe Ser Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Phe
    65   70   75   80

```
        Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                        85                  90                  95
1
        Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Pro
                    100             105                 110

        Val Thr Val Ser Ser
                    115
5

    (2) INFORMATION FOR SEQ ID NO:12:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 108 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear
10
        (ii) MOLECULE TYPE: peptide


        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

        Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
        1               5               10                  15
15      Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Arg Lys Tyr
                    20              25                  30
        Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ala Pro Lys Thr Leu Ile
                    35              40                  45
        Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
            50                  55                  60
20      Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
        65                  70                  75                  80
        Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln His Gly Glu Ser Pro Tyr
                        85                  90                  95
        Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Thr Arg
                    100             105
25
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 117 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10              15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr
            20                  25                  30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp Pro Lys Phe
    50                  55                  60

Gln Gly Arg Phe Thr Ile Ser Ala Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Pro
            100                 105                 110

Val Thr Val Ser Ser
            115
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 108 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

5

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Arg Lys Tyr
              20   25   30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
         35   40   45

Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln His Gly Glu Ser Pro Tyr
              85   90   95

10

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Thr Arg
         100   105

15

(2) INFORMATION FOR SEQ ID NO:15:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 7073 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: double
       (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: peptide

   (ix) FEATURE:
       (A) NAME/KEY: CDS
       (B) LOCATION: 61..717

   (ix) FEATURE:
       (A) NAME/KEY: CDS
       (B) LOCATION: 1111..1146

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        (ix) FEATURE:
             (A) NAME/KEY: CDS
1   (B) LOCATION: 1268..1594

        (ix) FEATURE:
             (A) NAME/KEY: CDS
             (B) LOCATION: 1692..2012

5   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

    GAATTCGCCT CCACCATGGA ATGGAGCTGG GTCTTTCTCT TCTTCTTGTC AGTAACTACA   60

    GGT GTA CAC TCA CAA GTT CAG CTG GTG GAG TCT GGA GGA GGA GTA GTA   108
    Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val
     1   5   10   15

    CAA CCT GGA AGG TCA CTG AGA CTG TCT TGT AAG GCT AGT GGA TTC AAT   156
10 Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn
                20   25   30

    ATC AAG GAC TAT TAT ATG CAC TGG GTC AGA CAA GCT CCT GGA AAA GGA   204
    Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly
             35   40   45

    CTC GAG TGG ATA GGT TTA ATT GAT CCT GAG AAT GGT AAC ACG ATA TAT   252
    Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr
15   50   55   60

    GAT CCC AAG TTC CAA GGA AGA TTC ATA ATT TCT GCA GAC AAC TCT AAG   300
    Asp Pro Lys Phe Gln Gly Arg Phe Ile Ile Ser Ala Asp Asn Ser Lys
     65   70   75   80

    AAT ACA CTG TTC CTG CAG ATG GAC TCA CTC AGA CCT GAG GAT ACA GCA   348
    Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala
                     85   90   95
20
    GTC TAC TTT TGT GCT AGA GAT AAC AGT TAT TAC TTC GAC TAC TGG GGC   396
    Val Tyr Phe Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly
                    100   105   110

    CAA GGA ACA CCA GTC ACC GTG AGC TCA GCT TCC ACC AAG GGC CCA TCC   444
    Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
                115   120   125

25 GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC GAG AGC ACA GCC   492
    Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
        130   135   140

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   GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG        540
   Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
1  145                 150                 155                 160

   TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT        588
   Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                     165                 170                 175

   GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG        636
5  Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
                     180                 185                 190

   CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC AAC GTA GAT CAC        684
   Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His
                 195                 200                 205

   AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT GGTGAGAGGC CAGCACAGGG      737
   Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val
10     210                 215

   CAGGGAGGGT GTCTGCTGGA AGCCAGGCTC AGCCCTCCTG CCTGGACGCA CCCCGGCTGT      797

   GCAGCCCCAG CCCAGGGCAG CAAGGCATGC CCCATCTGTC TCCTCACCCG GAGGCCTCTG      857

   ACCACCCCAC TCATGCTCAG GGAGAGGGTC TTCTGGATTT TTCCACCAGG CTCCGGGCAG      917

   CCACAGGCTG GATGCCCCTA CCCCAGGCCC TGCGCATACA GGGGCAGGTG CTGCGCTCAG      977
15
   ACCTGCCAAG AGCCATATCC GGGAGGACCC TGCCCCTGAC CTAAGCCCAC CCCAAAGGCC     1037

   AAACTCTCCA CTCCCTCAGC TCAGACACCT TCTCTCCTCC CAGATTCGAG TAACTCCCAA     1097

   TCTTCTCTCT GCA GAG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA        1146
                   Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro
                   1               5               10

20 GGTAAGCCAA CCCAGGCCTC GCCCTCCAGC TCAAGGCGGG ACAGGTGCCC TAGAGTAGCC     1206

   TGCATCCAGG GACAGGCCCC AGCCGGGTGC TGACGCATCC ACCTCCATCT CTTCCTCAGC     1266

   A CCT GAG TTC CTG GGG GGA CCA TCA GTC TTC CTG TTC CCC CCA AAA        1312
     Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
     1               5                   10                  15

   CCC AAG GAC ACT CTC ATG ATC TCC CGG ACC CCT GAG GTC ACG TGC GTG     1360
25 Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
               20                  25                  30
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     GTG GTG GAC GTG AGC CAG GAA GAC CCC GAG GTC CAG TTC AAC TGG TAC    1408
 1   Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
                 35                  40                  45

     GTG GAT GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG    1456
     Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
             50                  55                  60

     CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC ATG CAC    1504
 5   Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Met His
             65                  70                  75

     CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA    1552
     Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
     80                  85                  90                  95

     GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC AAA GCC AAA            1594
10   Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
                 100                 105

     GGTGGGACCC ACGGGGTGCG AGGGCCACAT GGACAGAGGT CAGCTCGGCC CACCCTCTGC   1654

     CCTGGGAGTG ACCGCTGTGC CAACCTCTGT CCCTACA GGG CAG CCC CGA GAG CCA   1709
                                             Gly Gln Pro Arg Glu Pro
                                             1                   5

     CAG GTG TAC ACC CTG CCC CCA TCC CAG GAG GAG ATG ACC AAG AAC CAG    1757
15   Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln
                 10                  15                  20

     GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAC CCC AGC GAC ATC GCC    1805
     Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
                 25                  30                  35

     GTG GAG TGG GAG AGT AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG    1853
20   Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
             40                  45                  50

     CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AGG CTA    1901
     Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu
     55                  60                  65                  70

     ACC GTG GAC AAG AGC AGG TGG CAG GAG GGG AAT GTC TTC TCA GTC TCC    1949
     Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Val Ser
                 75                  80                  85
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      GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACA CAG AAG AGC CTC TCC    1997
      Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
  1           90                      95                  100

      CTG TCT CTG GGT AAA TGAGTGCCAG GGCCGGCAAG CCCCCGCTCC CCGGGCTCTC    2052
      Leu Ser Leu Gly Lys
                  105

      GGGGTCGCGC GAGGATGCTT GGCACGTACC CCGTCTACAT ACTTCCCAGG CACCCAGCAT    2112

      GGAAATAAAG CACCCACCAC TGCCCTGGGC CCCTGTGAGA CTGTGATGGT TCTTTCCACG    2172

      GGTCAGGCCG AGTCTGAGGC CTGAGTGACA TGAGGGAGGC AGAGCGGGTC CCACTGTCCC    2232

      CACACTGGCC CAGGCTGTGC AGGTGTGCCT GGGCCACCTA GGGTGGGGCT CAGCCAGGGG    2292

      CTGCCCTCGG CAGGGTGGGG GATTTGCCAG CGTGGCCCTC CCTCCAGCAG CAGGACTCTA    2352

      GAGGATCATA ATCAGCCATA CCACATTTGT AGAGGTTTTA CTTGCTTTAA AAAACCTCCC    2412

      ACACCTCCCC CTGAACCTGA AACATAAAAT GAATGCAATT GTTGTTGTTA ACTTGTTTAT    2472

      TGCAGCTTAT AATGGTTACA AATAAAGCAA TAGCATCACA AATTTCACAA ATAAAGCATT    2532

      TTTTTCACTG CATTCTAGTT GTGGTTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG    2592

      GATCCTCTAC GCCGGACGCA TCGTGGCCGG CATCACCGGC GCCACAGGTG CGGTTGCTGG    2652

      CGCCTATATC GCCGACATCA CCGATGGGGA AGATCGGGCT CGCCACTTCG GGCTCATGAG    2712

      CGCTTGTTTC GGCGTGGGTA TGGTGGCAGG CCCGTGGCCG GGGGACTGTT GGGCGCCATC    2772

      TCCTTGCATG CACCATTCCT TGCGGCGGCG GTGCTCAACG GCCTCAACCT ACTACTGGGC    2832

      TGCTTCCTAA TGCAGGAGTC GCATAAGGGA GAGCGTCGAC CTCGGGCCGC GTTGCTGGCG    2892

      TTTTTCCATA GGCTCCGCCC CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG    2952

      TGGCGAAACC CGACAGGACT ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG    3012

      CGCTCTCCTG TTCCGACCCT GCCGCTTACC GGATACCTGT CCGCCTTTCT CCCTTCGGGA    3072

      AGCGTGGCGC TTTCTCAATG CTCACGCTGT AGGTATCTCA GTTCGGTGTA GGTCGTTCGC    3132

      TCCAAGCTGG GCTGTGTGCA CGAACCCCCC GTTCAGCCCG ACCGCTGCGC CTTATCCGGT    3192

      AACTATCGTC TTGAGTCCAA CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT    3252
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      GGTAACAGGA TTAGCAGAGC GAGGTATGTA GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG   3312
  1   CCTAACTACG GCTACACTAG AAGGACAGTA TTTGGTATCT GCGCTCTGCT GAAGCCAGTT   3372
      ACCTTCGGAA AAAGAGTTGG TAGCTCTTGA TCCGGCAAAC AAACCACCGC TGGTAGCGGT   3432
      GGTTTTTTTG TTTGCAAGCA GCAGATTACG CGCAGAAAAA AAGGATCTCA AGAAGATCCT   3492
      TTGATCTTTT CTACGGGGTC TGACGCTCAG TGGAACGAAA ACTCACGTTA AGGGATTTTG   3552
  5   GTCATGAGAT TATCAAAAAG GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTTT   3612
      AAATCAATCT AAAGTATATA TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT   3672
      GAGGCACCTA TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC   3732
      GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC AATGATACCG   3792
  10  CGAGACCCAC GCTCACCGGC TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC   3852
      GAGCGCAGAA GTGGTCCTGC AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG   3912
      GAAGCTAGAG TAAGTAGTTC GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA   3972
      GGCATCGTGG TGTCACGCTC GTCGTTTGGT ATGGCATCAT TCAGCTCCGG TTCCCAACGA   4032
      TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC CTTCGGTCCT   4092
  15  CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC TCATGGTTAT GGCAGCACTG   4152
      CATAATTCTC TTACTGTCAT GCCATCCGTA AGATGCTTTT CTGTGACTGG TGAGTACTCA   4212
      ACCAAGTCAT TCTGAGAATA GTGTATGCGG CGACCGAGTT GCTCTTGCCC GGCGTCAACA   4272
      CGGGATAATA CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT   4332
  20  TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT GTAACCCACT   4392
      CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA GCGTTTCTGG GTGAGCAAAA   4452
      ACAGGAAGGC AAAATGCCGC AAAAAAGGGA ATAAGGGCGA CACGGAAATG TTGAATACTC   4512
      ATACTCTTCC TTTTTCAATA TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA   4572
      TACATATTTG AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA   4632
  25  AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAACCTA TAAAAATAGG   4692
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      CGTATCACGA GGCCCTGATG GCTCTTTGCG GCACCCATCG TTCGTAATGT TCCGTGGCAC    4752
   1  CGACGACAAC CCTCAAGAGA AAATGTAATC ACACTGGCTC ACCTTCGGGT GGGCCTTTCT    4812
      GCGTTTATAA GGAGACACTT TATGTTTAAG AAGGTTGGTA AATTCCTTGC GGCTTTGGCA    4872
      GCCAAGCTAG AGATCTCTAG CTTCGTGTCA AGGACGGTGA CTGCAGTGAA TAATAAAATG    4932
      TGTGTTTGTC CGAAATACGC GTTTTGAGAT TTCTGTCGCC GACTAAATTC ATGTCGCGCG    4992
   5  ATAGTGGTGT TTATCGCCGA TAGAGATGGC GATATTGGAA AAATCGATAT TTGAAAATAT    5052
      GGCATATTGA AAATGTCGCC GATGTGAGTT TCTGTGTAAC TGATATCGCC ATTTTTCCAA    5112
      AAGTGATTTT TGGGCATACG CGATATCTGG CGATAGCGCT TATATCGTTT ACGGGGGATG    5172
      GCGATAGACG ACTTTGGTGA CTTGGGCGAT TCTGTGTGTC GCAAATATCG CAGTTTCGAT    5232
  10  ATAGGTGACA GACGATATGA GGCTATATCG CCGATAGAGG CGACATCAAG CTGGCACATG    5292
      GCCAATGCAT ATCGATCTAT ACATTGAATC AATATTGGCC ATTAGCCATA TTATTCATTG    5352
      GTTATATAGC ATAAATCAAT ATTGGCTATT GGCCATTGCA TACGTTGTAT CCATATCATA    5412
      ATATGTACAT TTATATTGGC TCATGTCCAA CATTACCGCC ATGTTGACAT TGATTATTGA    5472
      CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA TAGCCCATAT ATGGAGTTCC    5532
  15  GCGTTACATA ACTTACGGTA AATGGCCCGC CTGGCTGACC GCCCAACGAC CCCCGCCCAT    5592
      TGACGTCAAT AATGACGTAT GTTCCCATAG TAACGCCAAT AGGGACTTTC CATTGACGTC    5652
      AATGGGTGGA GTATTTACGG TAAACTGCCC ACTTGGCAGT ACATCAAGTG TATCATATGC    5712
      CAAGTACGCC CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT TATGCCCAGT    5772
  20  ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA CGTATTAGTC ATCGCTATTA    5832
      CCATGGTGAT GCGGTTTTGG CAGTACATCA ATGGGCGTGG ATAGCGGTTT GACTCACGGG    5892
      GATTTCCAAG TCTCCACCCC ATTGACGTCA ATGGGAGTTT GTTTTGGCAC CAAAATCAAC    5952
      GGGACTTTCC AAAATGTCGT AACAACTCCG CCCCATTGAC GCAAATGGGC GGTAGGCGTG    6012
      TACGGTGGGA GGTCTATATA AGCAGAGCTC GTTTAGTGAA CCGTCAGATC GCCTGGAGAC    6072
  25  GCCATCCACG CTGTTTTGAC CTCCATAGAA GACACCGGGA CCGATCCAGC CTCCGCGGCC    6132
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     GGGAACGGTG  CATTGGAACG  CGGATTCCCC  GTGCCAAGAG  TGACGTAAGT  ACCGCCTATA    6192
  1  GAGTCTATAG  GCCCACCCCC  TTGGCTTCTT  ATGCATGCTA  TACTGTTTTT  GGCTTGGGGT    6252
     CTATACACCC  CCGCTTCCTC  ATGTTATAGG  TGATGGTATA  GCTTAGCCTA  TAGGTGTGGG    6312
     TTATTGACCA  TTATTGACCA  CTCCCCTATT  GGTGACGATA  CTTTCCATTA  CTAATCCATA    6372
     ACATGGCTCT  TTGCCACAAC  TCTCTTTATT  GGCTATATGC  CAATACACTG  TCCTTCAGAG    6432
  5  ACTGACACGG  ACTCTGTATT  TTTACAGGAT  GGGGTCTCAT  TTATTATTTA  CAAATTCACA    6492
     TATACAACAC  CACCGTCCCC  AGTGCCCGCA  GTTTTTATTA  AACATAACGT  GGGATCTCCA    6552
     CGCGAATCTC  GGGTACGTGT  TCCGGACATG  GGCTCTTCTC  CGGTAGCGGC  GGAGCTTCTA    6612
     CATCCGAGCC  CTGCTCCCAT  CCCTCCAGCG  ACTCATGGTC  GCTCGGCAGC  TCCTTGCTCC    6672
 10  TAACAGTGGA  GGCCAGACTT  AGGCACAGCA  CGATGCCCAC  CACCACCAGT  GTGCCGCACA    6732
     AGGCCGTGGC  GGTAGGGTAT  GTGTCTGAAA  ATGAGCTCGG  GGAGCGGGCT  TGCACCGCTG    6792
     ACGCATTTGG  AAGACTTAAG  GCAGCGGCAG  AAGAAGATGC  AGGCAGCTGA  GTTGTTGTGT    6852
     TCTGATAAGA  GTCAGAGGTA  ACTCCCGTTG  CGGTGCTGTT  AACGGTGGAG  GGCAGTGTAG    6912
     TCTGAGCAGT  ACTCGTTGCT  GCCGCGCGCG  CCACCAGACA  TAATAGCTGA  CAGACTAACA    6972
 15  GACTGTTCCT  TTCCATGGGT  CTTTTCTGCA  GTCACCGTCC  TTGACACGAA  GCTTGGGCTG    7032
     CAGGTCGATC  GACTCTAGAG  GATCGATCCC  CGGGCGAGCT  C                         7073
```

        (2) INFORMATION FOR SEQ ID NO:16:

 20   (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 219 amino acids
                    (B) TYPE: amino acid
                    (D) TOPOLOGY: linear

              (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
 1   Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val
      1               5               10                  15

     Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn
                  20              25              30

     Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly
 5               35              40              45

     Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr
          50              55              60

     Asp Pro Lys Phe Gln Gly Arg Phe Ile Ile Ser Ala Asp Asn Ser Lys
     65              70              75              80

     Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala
10               85              90              95

     Val Tyr Phe Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly
              100             105             110

     Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
              115             120             125

     Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
15       130             135             140

     Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
     145             150             155             160

     Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                  165             170             175

     Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
20               180             185             190

     Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His
              195             200             205

     Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val
              210             215
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(2) INFORMATION FOR SEQ ID NO:17:

1   (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 12 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: protein

5   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro
 1   5   10

(2) INFORMATION FOR SEQ ID NO:18:

10   (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 109 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear

          (ii) MOLECULE TYPE: protein

15   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
 1   5   10   15

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
                20   25   30

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
20   35   40   45

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
             50   55   60

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Met His Gln
65   70   75   80

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
25   85   90   95

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     Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
 1           100             105
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(2) INFORMATION FOR SEQ ID NO:19:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 107 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
     Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
10    1           5               10          Pro Pro     15

     Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
                 20              25              30

     Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
             35              40              45

     Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
15       50              55              60

     Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
         65              70              75              80

     Asn Val Phe Ser Val Ser Val Met His Glu Ala Leu His Asn His Tyr
                 85              90              95

     Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
20           100             105
```

(2) INFORMATION FOR SEQ ID NO:20:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 7864 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: double
       (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

1   (ix) FEATURE:
              (A) NAME/KEY: CDS
              (B) LOCATION: 9..711

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

5   AATTCACCAT GGGTGTGCCA ACTCAGGTAT TAGGATTACT GCTGCTGTGG CTTACAGATG   60

    CAAGATGTGA TATCCAAATG ACACAATCTC CTTCTTCTCT AAGTGCTTCT GTCGGAGATA   120

    GAGTAACAAT TACATGTAAG GCGAGTCAGG ACATTAGAAA GTATTTAAAC TGGTATCAGC   180

    AAAAACCTGG GAAGGCTCCT AAGCTACTGA TTTATTATGC AACAAGTTTG GCAGATGGAG   240

    TACCTTCTAG ATTTTCTGGT TCTGGCTCTG GAACAGACTA CACATTCACA ATTTCTTCTC   300

10 TCCAACCTGA GGACATTGCT ACATACTACT GCCTACAACA TGGTGAGAGT CCGTATACAT   360

    TTGGACAAGG AACAAAACTA GAGATCACAA GAACTGTTGC GGCGCCGTCT GTCTTCATCT   420

    TCCCGCCATC TGATGAGCAG TTGAAATCTG GAACTGCCTC TGTTGTGTGC CTGCTGAATA   480

    ACTTCTATCC CAGAGAGGCC AAAGTACAGT GGAAGGTGGA TAACGCCCTC CAATCGGGTA   540

15 ACTCCCAGGA GAGTGTCACA GAGCAGGACA GCAAGGACAG CACCTACAGC CTCAGCAGCA   600

    CCCTGACGCT GAGCAAAGCA GACTACGAGA AACACAAAGT CTACGCCTGC GAAGTCACCC   660

    ATCAGGGCCT GAGCTCGCCC GTCACAAAGA GCTTCAACAG GGGAGAGTGT TAGAGGGAGA   720

    AGTGCCCCCA CCTGCTCCTC AGTTCCAGCC TGGGGATCAT AATCAGCCAT ACCACATTTG   780

    TAGAGGTTTT ACTTGCTTTA AAAAACCTCC CACACCTCCC CCTGAACCTG AAACATAAAA   840

20 TGAATGCAAT TGTTGTTGTT AACTTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA   900

    ATAGCATCAC AAATTTCACA AATAAAGCAT TTTTTTCACT GCATTCTAGT TGTGGTTTGT   960

    CCAAACTCAT CAATGTATCT TATCATGTCT GGATCCTCTA CGCCGGACGC ATCGTGGCCG   1020

    GCATCACCGG CGCCACAGGT GCGGTTGCTG CGCCTATAT CGCCGACATC ACCGATGGGG   1080

25 AAGATCGGGC TCGCCACTTC GGGCTCATGA GCGCTTGTTT CGGCGTGGGT ATGGTGGCAG   1140

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        GCCCGTGGCC GGGGGACTGT TGGGCGCCAT CTCCTTGCAT GCACCATTCC TTGCGGCGGC   1200
    1   GGTGCTCAAC GGCCTCAACC TACTACTGGG CTGCTTCCTA ATGCAGGAGT CGCATAAGGG   1260
        AGAGCGTCGA CCTCGGGCCG CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC CCCCTGACGA   1320
        GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC CCGACAGGAC TATAAAGATA   1380
    5   CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT GCGCTCTCCT GTTCCGACCC TGCCGCTTAC   1440
        CGGATACCTG TCCGCCTTTC TCCCTTCGGG AAGCGTGGCG CTTTCTCAAT GCTCACGCTG   1500
        TAGGTATCTC AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC   1560
        CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCA ACCCGGTAAG   1620
        ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG ATTAGCAGAG CGAGGTATGT   1680
   10   AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGGACAGT   1740
        ATTTGGTATC TGCGCTCTGC TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG   1800
        ATCCGGCAAA CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC   1860
        GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA   1920
   15   GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA TTATCAAAAA GGATCTTCAC   1980
        CTAGATCCTT TTAAATTAAA AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAAAC   2040
        TTGGTCTGAC AGTTACCAAT GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT   2100
        TCGTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT   2160
        ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA CGCTCACCGG CTCCAGATTT   2220
   20   ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA AGTGGTCCTG CAACTTTATC   2280
        CGCCTCCATC CAGTCTATTA ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA   2340
        TAGTTTGCGC AACGTTGTTG CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGG   2400
        TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT   2460
   25   GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA AGTTGGCCGC   2520
        AGTGTTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT   2580
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      AAGATGCTTT TCTGTGACTG GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG    2640
  1   GCGACCGAGT TGCTCTTGCC CGGCGTCAAC ACGGGATAAT ACCGCGCCAC ATAGCAGAAC    2700
      TTTAAAAGTG CTCATCATTG GAAAACGTTC TTCGGGGCGA AAACTCTCAA GGATCTTACC    2760
      GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC AACTGATCTT CAGCATCTTT    2820
      TACTTTCACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAAGGG    2880
  5   AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC CTTTTTCAAT ATTATTGAAG    2940
      CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA    3000
      ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGTCT AAGAAACCAT    3060
      TATTATCATG ACATTAACCT ATAAAAATAG GCGTATCACG AGGCCCTGAT GGCTCTTTGC    3120
 10   GGCACCCATC GTTCGTAATG TTCCGTGGCA CCGAGGACAA CCCTCAAGAG AAAATGTAAT    3180
      CACACTGGCT CACCTTCGGG TGGGCCTTTC TGCGTTTATA AGGAGACACT TTATGTTTAA    3240
      GAAGGTTGGT AAATTCCTTG CGGCTTTGGC AGCCAAGCTA GAGATCCGGC TGTGGAATGT    3300
      GTGTCAGTTA GGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT    3360
      GCATCTCAAT TAGTCAGCAA CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC    3420
 15   TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC    3480
      CCAGTTCCGC CCATTCTCCG CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG    3540
      AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG AGGCTTTTTT GGAGGCCTAG    3600
      GCTTTTGCAA AAAGCTAGCT TGGGGCCACC GCTCAGAGCA CCTTCCACCA TGGCCACCTC    3660
 20   AGCAAGTTCC CACTTGAACA AAAACATCAA GCAAATGTAC TTGTGCCTGC CCCAGGGTGA    3720
      GAAAGTCCAA GCCATGTATA TCTGGGTTGA TGGTACTGGA GAAGGACTGC GCTGCAAAAC    3780
      CCGCACCCTG GACTGTGAGC CCAAGTGTGT AGAAGAGTTA CCTGAGTGGA ATTTTGATGG    3840
      CTCTAGTACC TTTCAGTCTG AGGGCTCCAA CAGTGACATG TATCTCAGCC CTGTTGCCAT    3900
      GTTTCGGGAC CCCTTCCGCA GAGATCCCAA CAAGCTGGTG TTCTGTGAAG TTTTCAAGTA    3960
 25   CAACCGGAAG CCTGCAGAGA CCAATTTAAG CGCACTCGTGT AAACGGATAA TGGACATGGT    4020
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     GAGCAACCAG CACCCCTGGT TTGGAATGGA ACAGGAGTAT ACTCTGATGG GAACAGATGG    4080
  1  GCACCCTTTT GGTTGGCCTT CCAATGGCTT TCCTGGGCCC CAAGGTCCGT ATTACTGTGG    4140
     TGTGGGCGCA GACAAAGCCT ATGGCAGGGA TATCGTGGAG GCTCACTACC GCGCCTGCTT    4200
     GTATGCTGGG GTCAAGATTA CAGGAACAAA TGCTGAGGTC ATGCCTGCCC AGTGGGAACT    4260
  5  CCAAATAGGA CCCTGTGAAG GAATCCGCAT GGGAGATCAT CTCTGGGTGG CCCGTTTCAT    4320
     CTTNCATCGA GTATGTGAAG ACTTTGGGGT AATAGCAACC TTTGACCCCA AGCCCATTCC    4380
     TGGGAACTGG AATGGTGCAG GCTGCCATAC CAACTTTAGC ACCAAGGCCA TGCGGGAGGA    4440
     GAATGGTCTG AAGCACATCG AGGAGGCCAT CGAGAAACTA AGCAAGCGGC ACCGGTACCA    4500
     CATTCGAGCC TACGATCCCA AGGGGGGCCT GGACAATGCC CGTGGTCTGA CTGGGTTCCA    4560
 10  CGAAACGTCC AACATCAACG ACTTTTCTGC TGGTGTCGCC AATCGCAGTG CCAGCATCCG    4620
     CATTCCCCCG ACTGTCGGCC AGGAGAAGAA AGGTTACTTT GAAGACCGCG GCCCCTCTGC    4680
     CAATTGTGAC CCCTTTGCAG TGACAGAAGC CATCGTCCGC ACATGCCTTC TCAATGAGAC    4740
     TGGCCACGAG CCCTTCCAAT ACAAAAACTA ATTAGACTTT GAGTGATCTT GAGCCTTTCC    4800
 15  TAGTTCATCC CACCCCGCCC CAGAGAGATC TTTGTGAAGG AACCTTACTT CTGTGGTGTG    4860
     ACATAATTGG ACAAACTACC TACAGAGATT TAAAGCTCTA AGGTAAATAT AAAATTTTTA    4920
     AGTGTATAAT GTGTTAAACT ACTGATTCTA ATTGTTGTG TATTTTAGAT TCCAACCTAT    4980
     GGAACTGATG AATGGGAGCA GTGGTGGAAT GCCTTTAATG AGGAAAACCT GTTTTGCTCA    5040
     GAAGAAATGC CATCTAGTGA TGATGAGGCT ACTGCTGACT CTCAACATTC TACTCCTCCA    5100
 20  AAAAAGAAGA GAAAGGTAGA ACACCCCAAG GACTTTCCTT CAGAATTGCT AAGTTTTTTG    5160
     AGTCATGCTG TGTTTAGTAA TAGAACTCTT GCTTGCTTTG CTATTTACAC CACAAAGGAA    5220
     AAAGCTGCAC TGCTATACAA GAAAATTATG GAAAAATATT CTGTAACCTT TATAAGTAGG    5280
     CATAACAGTT ATAATCATAA CATACTGTTT TTTCTTACTC CACACAGGCA TAGAGTGTCT    5340
 25  GCTATTAATA ACTATGCTCA AAAATTGTGT ACCTTTAGCT TTTTAATTTG TAAAGGGGTT    5400
     AATAAGGAAT ATTTGATGTA TAGTGCCTAG ACTAGAGATC ATAATCAGCC ATACCACATT    5460
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      TGTAGAGGTT TTACTTCCTT TAAAAAACCT CCCACACCTC CCCCTGAACC TGAAACATAA    5520
 1    AATGAATGCA ATTGTTGTTG TTAACTTGTT TATTGCAGCT TATAATGGTT ACAAATAAAG    5580
      CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA CTGCATTCTA GTTGTGGTTT    5640
      GTCCAAACTC ATCAATGTAT CTTATCATGT CTGGATCTCT AGCTTCGTGT CAAGGACGGT    5700
      GACTGCAGTG AATAATAAAA TGTGTGTTTG TCCGAAATAC GCGTTTTGAG ATTTCTGTCG    5760
 5    CCTACTAAAT TCATGTCGCG CGATAGTGGT GTTTATCGCC GATAGAGATG GCGATATTGG    5820
      AAAAATCGAT ATTTGAAAAT ATGGCATATT GAAAATGTCG CCGATGTGAG TTTCTGTGTA    5880
      ACTGATATCG CCATTTTTCC AAAAGTGATT TTTGGGCATA CGCGATATCT GGCGATAGCG    5940
      CTTATATCGT TTACGGGGGA TGGCGATAGA CGACTTTGGT GACTTGGGCG ATTCTGTGTG    6000
 10   TCGCAAATAT CGCAGTTTCG ATATAGGTGA CAGACGATAT GAGGCTATAT CGCCGATAGA    6060
      GGCGACATCA AGCTGGCACA TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG    6120
      CCATTAGCCA TATTATTCAT TGGTTATATA GCATAAATCA ATATTGGCTA TTGGCCATTG    6180
      CATACGTTGT ATCCATATCA TAATATGTAC ATTTATATTG GCTCATGTCC AACATTACCG    6240
      CCATGTTGAC ATTGATTATT GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT   `6300
 15   CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA    6360
      CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA    6420
      ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTTAC GGTAAACTGC CCACTTGGCA    6480
      GTACATCAAG TGTATCATAT GCCAAGTACG CCCCCTATTG ACGTCAATGA CGGTAAATGG    6540
 20   CCCGCCTGGC ATTATGCCCA GTACATGACC TTATGGGACT TTCCTACTTG GCAGTACATC    6600
      TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT CAATGGGCGT    6660
      GGATAGCGGT TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT    6720
      TTGTTTTGGC ACCAAAATCA ACGGGACTTT CCAAAATGTC GTAACAACTC CGCCCCATTG    6780
 25   ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC TCGTTTAGTG    6840
      AACCGTCAGA TCGCCTGGAG ACGCCATCCA CGCTGTTTTG ACCTCCATAG AAGACACCGG    6900
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Inter   onal Application No
PC I/US 96/09287

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		CA-A-	2050479	22-06-91
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		DE-D-	69022982	16-11-95
		DE-T-	69022982	28-03-96
		EP-A-	0460167	11-12-91
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		EP-A-	0626390	30-11-94
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		WO-A-	9109966	11-07-91
		WO-A-	9109967	11-07-91
		GB-A,B	2246781	12-02-92
		GB-A,B	2246570	05-02-92
		GB-A,B	2268744	19-01-94
		GB-A,B	2268745	19-01-94
		JP-T-	4505398	24-09-92
		JP-T-	4506458	12-11-92
		JP-T-	5500312	28-01-93
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		US-A-	5223427	29-06-93
		AU-B-	605864	24-01-91
		AU-A-	1627488	02-11-88
		EP-A-	0309548	05-04-89

INTERNATIONAL SEARCH REPORT

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:   31-35
   because they relate to subject matter not required to be searched by this Authority, namely:
   Remark: Although claims 31-35 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   [ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF CRYSTAL GROWTH, vol. 122, no. 1-4, August 1992, AMSTERDAM, NL, pages 253-264, XP002015918 W. RUF ET AL.: "Purification, sequence and crystallization of an anti-tissue factor Fab and its use for the crystallization of tissue factor." see abstract see table 1 -----	1-37

1

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C12N15/13   C07K16/36   C07K16/46   A61K39/395   //C12N5/10,
         C12N15/85

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C12N   C07K   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category°	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 91 09968 A (CELLTECH LIMITED) 11 July 1991 see examples see claims ---	1-37
Y	WO 88 07543 A (SCRIPPS CLINIC AND RESEARCH FOUNDATION) 6 October 1988 see claims ---	1-37
A	WO 94 11029 A (THE SCRIPPS RESEARCH INSTITUTE ET AL.) 26 May 1994 see claims ---	1-37
A	WO 94 05328 A (THE SCRIPPS RESEARCH INSTITUTE) 17 March 1994 see examples see claims ---	1-37

-/--

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family
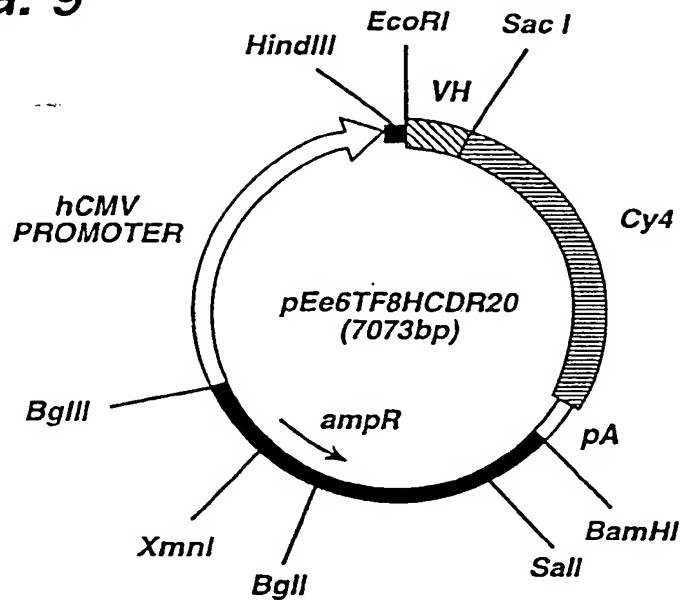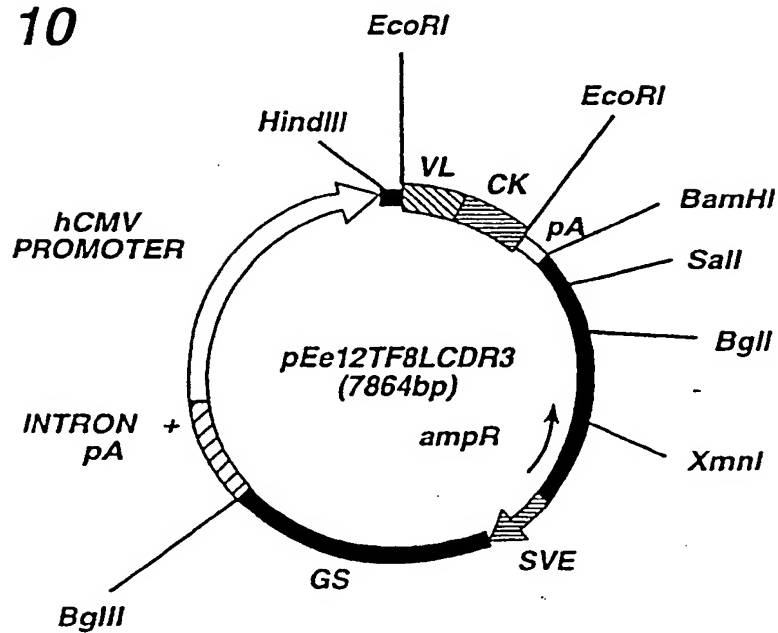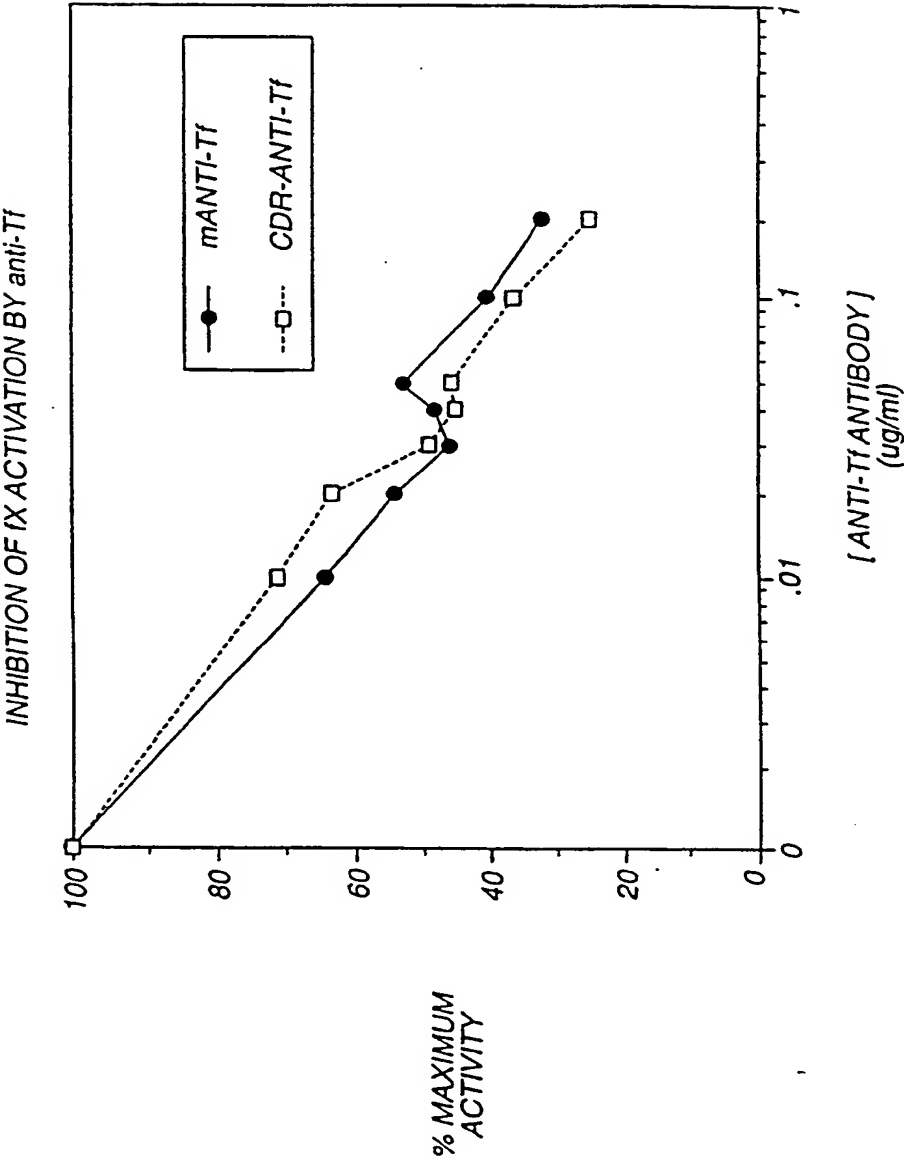
Date of the actual completion of the international search	Date of mailing of the international search report
15 October 1996	0 8. 11. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

page 1 of 2

FIG. 9

FIG. 10

FIG. 8

INHIBITION OF IX ACTIVATION BY anti-Tf

mANTI-Tf

CDR-ANTI-Tf

[ANTI-Tf ANTIBODY]
(ug/ml)

% MAXIMUM
ACTIVITY

FIG. 7

anti-TF COMPETITION ASSAY

ChL X ChH
LCDR3 X HCDR20

O.D. 490/650

ANTIBODY CONC. (ng/ml)

anti-TF BINDING ASSAY
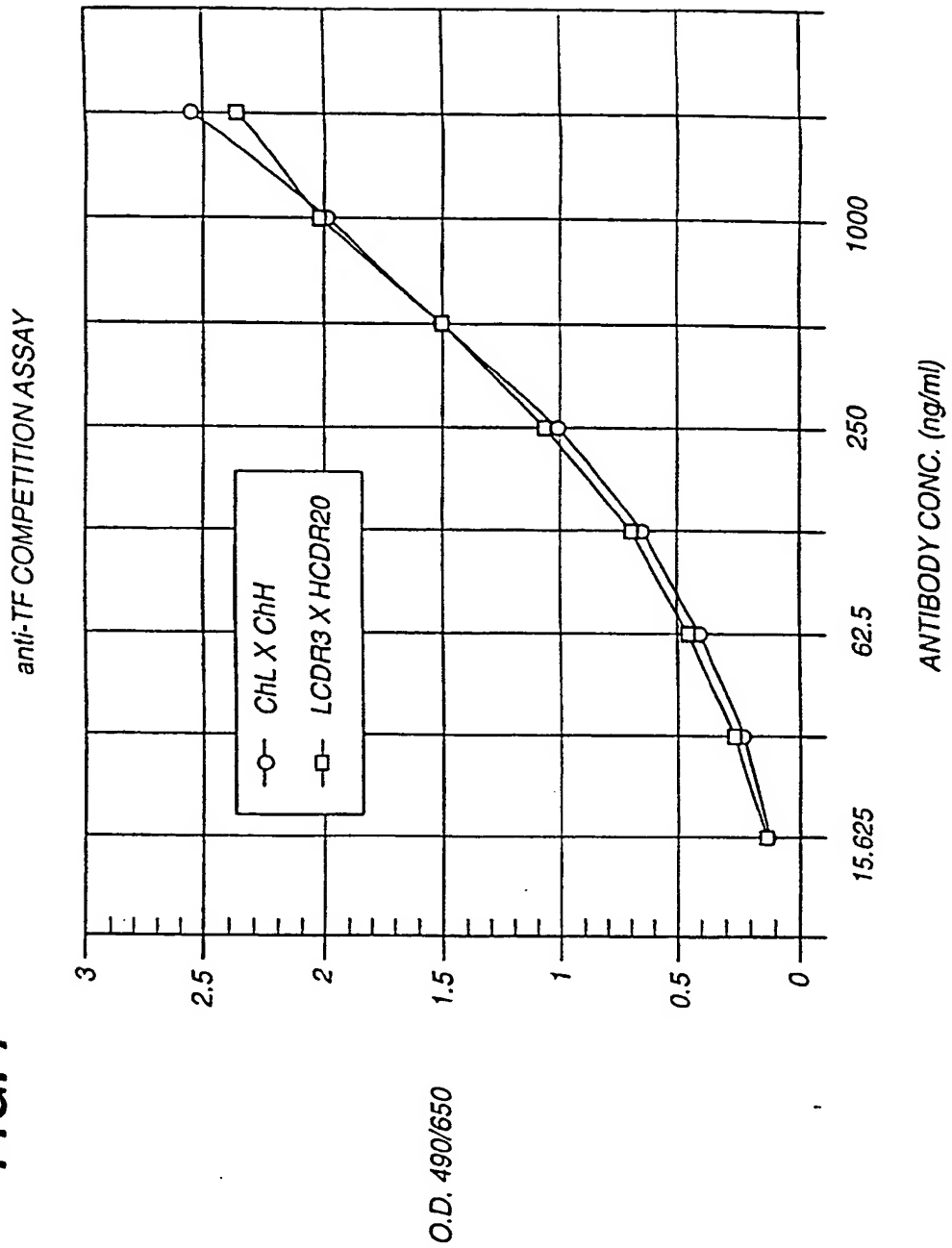
FIG. 6
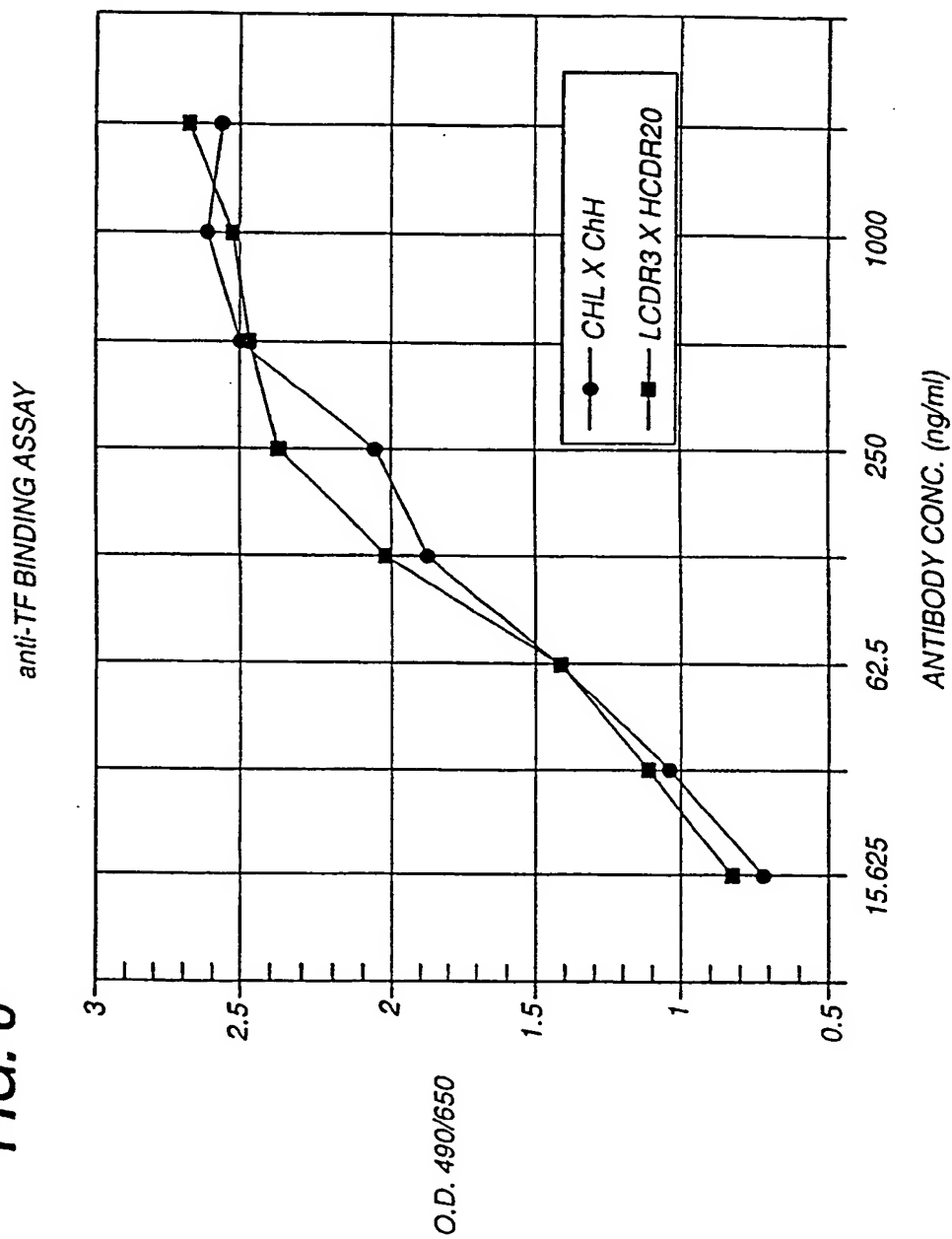
FIG. 5 O

```
    7830            7840            7850            7860
     •               •               •               •
CGA TCG ACT CTA GAG GAT CGA TCC CCG GGC GAG CTC G
GCT AGC TGA GAT CTC CTA GCT AGG GGC CCG CTC GAG C
```

FIG. 5 N

```
        7260            7270          7280         7290
         •               •             •            •
   TCG GGT CTC ATT TAT TAT TTA CAA ATT CAC ATA TAC AAC ACC ACC GTC
   ACC CCA GAG TAA ATA ATA AAT GTT TAA GTG TAT ATG TTG TGG TGG CAG

    7300         7310          7320         7330          7340
     •            •             •            •             •
   CCC AGT GCC CGC AGT TTT TAT TAA ACA TAA CGT GGG ATC TCC ACG CGA
   GGG TCA CGG GCG TCA AAA ATA ATT TGT ATT GCA CCC TAG AGG TCC GCT

    7350         7360          7370         7380          7390
     •            •             •            •             •
   ATC TCG GGT ACG TGT TCC GGA CAT GGG CTC TTC TCC GGT AGC GGC GGA
   TAG AGC CCA TGC ACA AGG CCT GTA CCC GAG AAG AGG CCA TCG CCG CCT

      7400         7410          7420         7430          7440
       •            •             •            •             •
   GCT TCT ACA TCC GAG CCC TGC TCC CAT GCC TCC AGC GAC TCA TGG TCG
   CGA AGA TGT AGG CTC GGG ACG AGG GTA CGG AGG TCG CTG AGT ACC AGC

        7450         7460          7470         7480          7490
         •            •             •            •             •
   CTC GGC AGC TCC TTG CTC CTA ACA GTG GAG GCC AGA CTT AGG CAC AGC
   GAG CCG TCG AGG AAC GAG GAT TGT CAC CTC CGG TCT GAA TCC GTG TCG

        7500         7510          7520         7530
         •            •             •            •
   ACG ATG CCC ACC ACC ACC AGT GTG CCG CAC AAG GCC GTG GCG GTA GGG
   TGC TAC GGG TGG TGG TGG TCA CAC GGC GTG TTC CGG CAC CGC CAT CCC

    7540         7550          7560          7570          7580
     •            •             •             •             •
   TAT GTG TCT GAA AAT GAG CTC GGG GAG CGG GCT TGC ACC GCT GAC GCA
   ATA CAC AGA CTT TTA CTC GAG CCC CTC GCC CGA ACG TGG CGA CTG CGT

    7590         7600          7610          7620          7630
     •            •             •             •             •
   TTT GGA AGA CTT AAG GCA GCG GCA GAA GAA GAT GCA GGC AGC TGA GTT
   AAA CCT TCT GAA TTC CGT CGC CGT CTT CTT CTA CGT CCG TCG ACT CAA

        7640         7650          7660         7670          7680
         •            •             •            •             •
   GTT GTG TTC TGA TAA GAG TCA GAG GTA ACT CCC GTT GCG GTC CTG TTA
   CAA CAC AAG ACT ATT CTC AGT CTC CAT TGA GGG CAA CGC CAC GAC AAT

        7690         7700          7710         7720          7730
         •            •             •            •             •
   ACG GTG GAG GGC AGT GTA GTC TGA GCA GTA CTC GTT GCT GCC GCG CGC
   TGC CAC CTC CCG TCA CAT CAG ACT CGT CAT GAG CAA CGA CGG CGC GCG

           7740         7750          7760         7770
            •            •             •            •
   GCC ACC AGA CAT AAT AGC TGA CAG ACT AAC AGA CTG TTC CTT TCC ATG
   CGG TGG TCT GTA TTA TCG ACT GTC TGA TTG TCT GAC AAG GAA AGG TAC

    7780         7790          7800         7810          7820
     •            •             •            •             •
   GGT CTT TTC TGC AGT CAC CGT CCT TGA CAC GAA GCT TCG GCT GCA GGT
   CCA GAA AAG ACG TCA GTG GCA GGA ACT GTG CTT CGA ACC CGA CGT CCA
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FIG. 5 M

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      6680         6690         6700         6710         6720
       •            •            •            •            •
TCA CGG GGA TTT CCA AGT CTC CAC CCC ATT GAC GTC AAT GGG AGT TTG
AGT GCC CCT AAA GGT TCA GAG GTG GGG TAA CTG CAG TTA CCC TCA AAC

         6730         6740         6750         6760         6770
          •            •            •            •            •
TTT TGG CAC CAA AAT CAA CGG GAC TTT CCA AAA TGT CGT AAC AAC TCC
AAA ACC GTG GTT TTA GTT GCC CTG AAA GGT TTT ACA GCA TTG TTG AGG

          6780         6790         6800         6810
           •            •            •            •
GCC CCA TTG ACG CAA ATG GGC GGT AGG CGT GTA CGG TGG GAG GTC TAT
CGG GGT AAC TGC GTT TAC CCG CCA TCC GCA CAT GCC ACC CTC CAG ATA

   6820         6830         6840         6850         6860
    •            •            •            •            •
ATA AGC AGA GCT CGT TTA GTG AAC CGT CAG ATC GCC TGG AGA CGC CAT
TAT TCG TCT CGA GCA AAT CAC TTG GCA GTC TAG CGG ACC TCT GCG GTA

  6870         6880         6890         6900         6910
   •            •            •            •            •
CCA CGC TGT TTT GAC CTC CAT AGA AGA CAC CGG GAC CGA TCC AGC CTC
GGT GCG ACA AAA CTG GAG GTA TCT TCT GTG GCC CTG GCT AGG TCG GAG

     6920         6930         6940         6950         6960
      •            •            •            •            •
CGC GGC CCG GAA CGG TGC ATT GGA ACG CGG ATT CCC CGT GCC AAG AGT
GCG CCG GCC CTT GCC ACG TAA CCT TGC GCC TAA GGG GCA CGG TTC TCA

       6970         6980         6990         7000         7010
        •            •            •            •            •
GAC GTA AGT ACC GCC TAT AGA GTC TAT AGG CCC ACC CCC TTG GCT TCT
CTG CAT TCA TGG CGG ATA TCT CAG ATA TCC GGG TGG GGG AAC CGA AGA

         7020         7030         7040         7050
          •            •            •            •
TAT GCA TGC TAT ACT GTT TTT CGC TTG GGG TCT ATA CAC CCC CGC TTC
ATA CGT ACG ATA TGA CAA AAA GCG AAC CCC AGA TAT GTG GGG GCG AAG

  7060         7070         7080         7090         7100
   •            •            •            •            •
CTC ATG TTA TAG GTG ATG GTA TAG CTT AGC CTA TAG GTC TGG GTT ATT
GAG TAC AAT ATC CAC TAC CAT ATC GAA TCG GAT ATC CAC ACC CAA TAA

 7110         7120         7130         7140         7150
  •            •            •            •            •
GAC CAT TAT TGA CCA CTC CCC TAT TGG TGA CGA TAC TTT CCA TTA CTA
CTG GTA ATA ACT GGT GAG GGG ATA ACC ACT GCT ATG AAA GGT AAT GAT

    7160         7170         7180         7190         7200
     •            •            •            •            •
ATC CAT AAC ATG GCT CTT TCC CAC AAC TCT CTT TAT TGG CTA TAT GCC
TAG GTA TTG TAC CGA GAA ACG GTG TTG AGA GAA ATA ACC GAT ATA CGG

      7210         7220         7230         7240         7250
       •            •            •            •            •
AAT ACA CTG TCC TTC AGA GAC TGA CAC GGA CTC TGT ATT TTT ACA GGA
TTA TGT GAC AGG AAG TCT CTG ACT GTG CCT GAG ACA TAA AAA TGT CCT
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FIG. 5 L

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  6100        6110        6120        6130        6140
   .           .           .           .           .
 TAT ACA TTG AAT CAA TAT TGG CCA TTA GCC ATA TTA TTC ATT GGT TAT
 ATA TGT AAC TTA GTT ATA ACC GGT AAT CGG TAT AAT AAG TAA CCA ATA

    6150        6160        6170        6180        6190
     .           .           .           .           .
 ATA GCA TAA ATC AAT ATT GGC TAT TGG CCA TTG CAT ACG TTG TAT CCA
 TAT CGT ATT TAG TTA TAA CCG ATA ACC GGT AAC GTA TGC AAC ATA GGT

     6200        6210        6220        6230        6240
      .           .           .           .           .
 TAT CAT AAT ATG TAC ATT TAT ATT GGC TCA TGT CCA ACA TTA CCG CCA
 ATA GTA TTA TAC ATG TAA ATA TAA CCG AGT ACA GGT TGT AAT GGC GGT

      6250        6260        6270        6280        6290
       .           .           .           .           .
 TGT TGA CAT TGA TTA TTG ACT AGT TAT TAA TAG TAA TCA ATT ACG GGG
 ACA ACT GTA ACT AAT AAC TGA TCA ATA ATT ATC ATT AGT TAA TGC CCC

       6300        6310        6320        6330
        .           .           .           .
 TCA TTA GTT CAT AGC CCA TAT ATG GAC TTC CGC GTT ACA TAA CTT ACG
 AGT AAT CAA GTA TCG GGT ATA TAC CTC AAG GCG CAA TGT ATT GAA TGC

  6340        6350        6360        6370        6380
   .           .           .           .           .
 GTA AAT GGC CCG CCT GGC TGA CCG CCC AAC GAC CCC CGC CCA TTG ACG
 CAT TTA CCG GGC GGA CCG ACT GGC GGG TTG CTG GGG GCG GGT AAC TGC

  6390        6400        6410        6420        6430
   .           .           .           .           .
 TCA ATA ATG ACG TAT GTT CCC ATA GTA ACG CCA ATA GGG ACT TTC CAT
 AGT TAT TAC TGC ATA CAA GGG TAT CAT TGC GGT TAT CCC TGA AAG GTA

    6440        6450        6460        6470        6480
     .           .           .           .           .
 TGA CGT CAA TGG GTG GAG TAT TTA CGG TAA ACT GCC CAC TTC GCA GTA
 ACT GCA GTT ACC CAC CTC ATA AAT GCC ATT TGA CGG GTG AAC CGT CAT

    6490        6500        6510        6520        6530
     .           .           .           .           .
 CAT CAA GTG TAT CAT ATG CCA AGT ACG CCC CCT ATT GAC GTC AAT GAC
 GTA GTT CAC ATA GTA TAC GGT TCA TGC GGG GGA TAA CTG CAG TTA CTG

      6540        6550        6560        6570
       .           .           .           .
 GGT AAA TGG CCC GCC TGG CAT TAT GCC CAG TAC ATG ACC TTA TGG GAC
 CCA TTT ACC GGG CGG ACC GTA ATA CGG GTC ATG TAC TGG AAT ACC CTG

  6580        6590        6600        6610        6620
   .           .           .           .           .
 TTT CCT ACT TGG CAG TAC ATC TAC GTA TTA GTC ATC GCT ATT ACC ATG
 AAA GGA TGA ACC GTC ATG TAG ATG CAT AAT CAG TAG CGA TAA TGG TAC

  6630        6640        6650        6660        6670
   .           .           .           .           .
 GTG ATG CGG TTT TGG CAG TAC ATC AAT GGG CGT GGA TAG CGG TTT GAC
 CAC TAC GCC AAA ACC GTC ATG TAG TTA CCC GCA CCT ATC GCC AAA CTG
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FIG. 5 K

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           5530              5540              5550              5560              5570
            •                 •                 •                 •                 •
GAA TGC AAT TGT TGT TGT TAA CTT GTT TAT TGC AGC TTA TAA TGG TTA
CTT ACG TTA ACA ACA ACA ATT GAA CAA ATA ACG TCG AAT ATT ACC AAT

           5580              5590              5600              5610
            •                 •                 •                 •
CAA ATA AAG CAA TAG CAT CAC AAA TTT CAC AAA TAA AGC ATT TTT TTC
GTT TAT TTC GTT ATC GTA GTG TTT AAA GTG TTT ATT TCG TAA AAA AAG

     5620         5630         5640         5650         5660
      •            •            •            •            •
ACT GCA TTC TAG TTG TGG TTT GTC CAA ACT CAT CAA TGT ATC TTA TCA
TGA CGT AAG ATC AAC ACC AAA CAG GTT TGA GTA GTT ACA TAG AAT AGT

     5670         5680         5690         5700         5710
      •            •            •            •            •
TGT CTG GAT CTC TAG CTT CGT GTC AAG GAC GGT GAC TGC AGT GAA TAA
ACA GAC CTA GAG ATC GAA GCA CAG TTC CTG CCA CTG ACG TCA CTT ATT

           5720         5730         5740         5750         5760
            •            •            •            •            •
TAA AAT GTG TGT TTG TCC GAA ATA CGC GTT TTG AGA TTT CTG TCG CCG
ATT TTA CAC ACA AAC AGG CTT TAT GCG CAA AAC TCT AAA GAC AGC GGC

           5770         5780         5790         5800         5810
            •            •            •            •            •
ACT AAA TTC ATG TCG CGC GAT AGT GGT GTT TAT CGC CGA TAG AGA TGG
TGA TTT AAG TAC AGC GCG CTA TCA CCA CAA ATA GCG GCT ATC TCT ACC

           5820         5830         5840         5850
            •            •            •            •
CGA TAT TGG AAA AAT CGA TAT TTG AAA ATA TGG CAT ATT GAA AAT GTC
GCT ATA ACC TTT TTA GCT ATA AAC TTT TAT ACC GTA TAA CTT TTA CAG

     5860         5870    ·    5880         5890         5900
      •            •            •            •            •
GCC GAT GTG AGT TTC TGT GTA ACT GAT ATC GCC ATT TTT CCA AAA GTG
CGG CTA CAC TCA AAG ACA CAT TGA CTA TAG CGG TAA AAA GGT TTT CAC

     5910         5920         5930         5940         5950
      •            •            •            •            •
ATT TTT GGG CAT ACG CGA TAT CTG GCG ATA GCG CTT ATA TCG TTT ACG
TAA AAA CCC GTA TGC GCT ATA GAC CGC TAT CGC GAA TAT AGC AAA TGC

     5960         5970         5980         5990         6000
      •            •            •            •            •
GGG GAT GGC GAT AGA CGA CTT TGG TGA CTT GGG CGA TTC TGT GTG TCG
CCC CTA CCG CTA TCT GCT GAA ACC ACT GAA CCC GCT AAG ACA CAC AGC

           6010         6020         6030         6040         6050
            •            •            •            •            •
CAA ATA TCG CAG TTT CGA TAT AGG TGA CAG ACG ATA TGA GGC TAT ATC
GTT TAT AGC GTC AAA GCT ATA TCC ACT GTC TGC TAT ACT CCG ATA TAG

           6060         6070         6080         6090
            •            •            •            •
GCC GAT AGA GGC GAC ATC AAG CTG GCA CAT GGC CAA TGC ATA TCG ATC
CGG CTA TCT CCG CTG TAG TTC GAC CGT GTA CCG GTT ACG TAT AGC TAG
```

FIG. 5 J

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      4950          4960          4970          4980          4990
       •            •             •             •             •
  CTA ATT GTT TGT GTA TTT TAG ATT CCA ACC TAT GGA ACT GAT GAA TGG
  GAT TAA CAA ACA CAT AAA ATC TAA GGT TGG ATA CCT TGA CTA CTT ACC


      5000          5010          5020          5030          5040
       •            •             •             •             •
  GAG CAG TGG TGG AAT GCC TTT AAT GAG GAA AAC CTG TTT TGC TCA GAA
  CTC GTC ACC ACC TTA CGG AAA TTA CTC CTT TTG GAC AAA ACG AGT CTT


       5050          5060          5070          5080          5090
        •            •             •             •             •
  GAA ATG CCA TCT AGT GAT GAT GAG GCT ACT GCT GAC TCT CAA CAT TCT
  CTT TAC GGT AGA TCA CTA CTA CTC CGA TGA CGA CTG AGA GTT GTA AGA


        5100          5110          5120          5130
         •            •             •             •
  ACT CCT CCA AAA AAG AAG AGA AAG GTA GAA GAC CCC AAG GAC TTT CCT
  TGA GGA GGT TTT TTC TTC TCT TTC CAT CTT CTG GGG TTC CTG AAA GGA


  5140          5150          5160          5170          5180
   •            •             •             •             •
  TCA GAA TTG CTA AGT TTT TTG AGT CAT GCT GTG TTT AGT AAT AGA ACT
  AGT CTT AAC GAT TCA AAA AAC TCA GTA CGA CAC AAA TCA TTA TCT TGA


  5190          5200          5210          5220          5230
   •            •             •             •             •
  CTT GCT TGC TTT GCT ATT TAC ACC ACA AAG GAA AAA GCT GCA CTG CTA
  GAA CGA ACG AAA CGA TAA ATG TGG TGT TTC CTT TTT CGA CGT GAC GAT


       5240          5250          5260          5270          5280
        •            •             •             •             •
  TAC AAG AAA ATT ATG GAA AAA TAT TCT GTA ACC TTT ATA AGT AGG CAT
  ATG TTC TTT TAA TAC CTT TTT ATA AGA CAT TGG AAA TAT TCA TCC GTA


        5290          5300          5310          5320          5330
         •            •             •             •             •
  AAC AGT TAT AAT CAT AAC ATA CTG TTT TTT CTT ACT CCA CAC AGG CAT
  TTG TCA ATA TTA GTA TTG TAT GAC AAA AAA GAA TGA GGT GTG TCC GTA


         5340          5350          5360          5370
          •            •             •             •
  AGA GTG TCT GCT ATT AAT AAC TAT GCT CAA AAA TTG TGT ACC TTT AGC
  TCT CAC AGA CGA TAA TTA TTG ATA CGA GTT TTT AAC ACA TGG AAA TCG


  5380          5390          5400          5410          5420
   •            •             •             •             •
  TTT TTA ATT TGT AAA GGG GTT AAT AAG GAA TAT TTG ATG TAT AGT GCC
  AAA AAT TAA ACA TTT CCC CAA TTA TTC CTT ATA AAC TAC ATA TCA CGG


  5430          5440          5450          5460          5470
   •            •             •             •             •
  TTG ACT AGA GAT CAT AAT CAG CCA TAC CAC ATT TGT AGA GGT TTT ACT
  AAC TGA TCT CTA GTA TTA GTC GGT ATG GTG TAA ACA TCT CCA AAA TGA


       5480          5490          5500          5510          5520
        •            •             •             •             •
  TGC TTT AAA AAA CCT CCC ACA CCT CCC CCT GAA CCT GAA ACA TAA AAT
  ACG AAA TTT TTT GGA GGG TGT GGA GGG GGA CTT GGA CTT TGT ATT TTA
```

FIG. 5 I

```
         4380              4390              4400              4410
          •                 •                 •                 •
GCC CAT TCC TGG GAA CTG GAA TGG TGC AGG CTG CCA TAC CAA CTT TAG
CGG GTA AGG ACC CTT GAC CTT ACC ACG TCC GAC GGT ATG GTT GAA ATC

   4420          4430              4440              4450          4460
    •             •                 •                 •             •
CAC CAA GGC CAT GCG GGA GGA GAA TGG TCT GAA GCA CAT CGA GGA GGC
GTG GTT CCG GTA CGC CCT CCT CTT ACC AGA CTT CGT GTA GCT CCT CCG

   4470              4480          4490          4500          4510
    •                 •             •             •             •
CAT CGA GAA ACT AAG CAA GCG GCA CCG GTA CCA CAT TCG AGC CTA CGA
GTA GCT CTT TGA TTC GTT CGC CGT GGC CAT GGT GTA AGC TCG GAT GCT

       4520          4530              4540          4550          4560
        •             •                 •             •             •
TCC CAA GGG GGG CCT GGA CAA TGC CCG TGG TCT GAC TGG GTT CCA CGA
AGG GTT CCC CCC GGA CCT GTT ACG GGC ACC AGA CTG ACC CAA GGT GCT

       4570          4580          4590          4600          4610
        •             •             •             •             •
AAC GTC CAA CAT CAA CGA CTT TTC TGC TGG TGT CGC CAA TCG CAG TGC
TTG CAG GTT GTA GTT GCT GAA AAG ACG ACC ACA GCG GTT AGC GTC ACG

           4620          4630          4640          4650
            •             •             •             •
CAG CAT CCG CAT TCC CCG GAC TGT CCG CCA GGA GAA GAA AGG TTA CTT
GTC GTA GGC GTA AGG GGC CTG ACA GCC GGT CCT CTT CTT TCC AAT GAA

   4660          4670              4680          4690          4700
    •             •                 •             •             •
TGA AGA CCG CGG CCC CTC TGC CAA TTG TGA CCC CTT TGC AGT GAC AGA
ACT TCT GGC GCC GGG GAG ACG GTT AAC ACT GGG GAA ACG TCA CTG TCT

   4710          4720          4730          4740          4750
    •             •             •             •             •
AGC CAT CGT CCG CAC ATG CCT TCT CAA TGA GAC TGG CCA CGA GCC CTT
TCG GTA GCA GGC GTG TAC GGA AGA GTT ACT CTG ACC GGT GCT CGG GAA

       4760          4770          4780          4790          4800
        •             •             •             •             •
CCA ATA CAA AAA CTA ATT AGA CTT TGA GTG ATC TTG AGC CTT TCC TAG
GGT TAT GTT TTT GAT TAA TCT GAA ACT CAC TAG AAC TCG GAA AGG ATC

       4810          4820          4830          4840          4850
        •             •             •             •             •
TTC ATC CCA CCC CGC CCC AGA GAG ATC TTT GTG AAG GAA CCT TAC TTC
AAG TAG GGT GGG GCG GGG TCT CTC TAG AAA CAC TTC CTT GGA ATG AAG

       4860          4870          4880          4890
        •             •             •             •
TGT CGT GTG ACA TAA TTC GAC AAA CTA CCT ACA GAG ATT TAA AGC TCT
ACA CCA CAC TGT ATT AAC CTG TTT GAT GGA TGT CTC TAA ATT TCG AGA

   4900          4910          4920          4930          4940
    •             •             •             •             •
AAG GTA AAT ATA AAA TTT TTA AGT GTA TAA TGT GTT AAA CTA CTG ATT
TTC CAT TTA TAT TTT AAA AAT TCA CAT ATT ACA CAA TTT GAT GAC TAA
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FIG. 5 H

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         3800            3810            3820            3830            3840
          •               •               •               •               •
TGA GCC CAA GTG TGT AGA AGA GTT ACC TGA GTG GAA TTT TGA TGG CTC
ACT CGG GTT CAC ACA TCT TCT CAA TGG ACT CAC CTT AAA ACT ACC GAG

         3850            3860            3870            3880            3890
          •               •               •               •               •
TAG TAC CTT TCA GTC TGA GGG CTC CAA CAG TGA CAT GTA TCT CAG CCC
ATC ATG GAA AGT CAG ACT CCC GAG GTT GTC ACT GTA CAT AGA GTC GGG

         3900            3910            3920            3930
          •               •               •               •
TGT TGC CAT GTT TCG GGA CCC CTT CCG CAG AGA TCC CAA CAA GCT GGT
ACA ACG GTA CAA AGC CCT GGG GAA GGC GTC TCT AGG GTT GTT CGA CCA

   3940            3950            3960            3970            3980
    •               •               •               •               •
GTT CTG TGA AGT TTT CAA GTA CAA CCG GAA GCC TGC AGA GAC CAA TTT
CAA GAC ACT TCA AAA GTT CAT GTT GGC CTT CGG ACG TCT CTC GTT AAA

   3990            4000            4010            4020            4030
    •               •               •               •               •
AAG GCA CTC GTG TAA ACG GAT AAT GGA CAT GGT GAG CAA CCA GCA CCC
TTC CGT GAG CAC ATT TGC CTA TTA CCT GTA CCA CTC GTT GGT CGT GGG

       4040            4050            4060            4070            4080
        •               •               •               •               •
CTG GTT TGG AAT GGA ACA GGA GTA TAC TCT GAT GGG AAC AGA TGG GCA
GAC CAA ACC TTA CCT TGT CCT CAT ATG AGA CTA CCC TTG TCT ACC CGT

         4090            4100            4110            4120            4130
          •               •               •               •               •
CCC TTT TGG TTG GCC TTC CAA TGG CTT TCC TGG GCC CCA AGG TCC GTA
GGG AAA ACC AAC CGG AAG GTT ACC GAA AGG ACC CGG GGT TCC AGG CAT

           4140            4150            4160            4170
            •               •               •               •
TTA CTG TGG TGT GGG CGC AGA CAA AGC CTA TGG CAG GGA TAT CGT GGA
AAT GAC ACC ACA CCC GCG TCT GTT TCG GAT ACC GTC CCT ATA GCA CCT

   4180            4190            4200            4210            4220
    •               •               •               •               •
CGC TCA CTA CCG CGC CTC CTT GTA TGC TGG GGT CAA GAT TAC AGG AAC
CCG AGT GAT GGC GCG GAG GAA CAT ACG ACC CCA GTT CTA ATG TCC TTG

   4230            4240            4250            4260            4270
    •               •               •               •               •
AAA TGC TGA GGT CAT CCC TGC CCA GTG GGA ACT CCA AAT AGG ACC CTG
TTT ACG ACT CCA GTA CGG ACG GGT CAC CCT TGA GGT TTA TCC TGG GAC

       4280            4290            4300            4310            4320
        •               •               •               •               •
TGA AGG AAT CCG CAT GGG AGA TCA TCT CTG GGT GGC CCG TTT CAT CTT
ACT TCC TTA GGC GTA CCC TCT AGT AGA GAC CCA CCG GGC AAA GTA GAA

         4330            4340            4350            4360            4370
          •               •               •               •               •
NCA TCG AGT ATG TGA AGA CTT TGG GGT AAT AGC AAC CTT TGA CCC CAA
NGT AGC TCA TAC ACT TCT GAA ACC CCA TTA TCG TTG GAA ACT GGG GTT
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FIG. 5 G

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    3220           3230           3240           3250           3260
     *             *              *              *              *
 AAG GAG ACA CTT TAT GTT TAA GAA GGT TGG TAA ATT CCT TGC GGC TTT
 TTC CTC TGT GAA ATA CAA ATT CTT CCA ACC ATT TAA GGA ACG CCG AAA

    3270           3280           3290           3300           3310
     *             *              *              *              *
 GGC AGC CAA GCT AGA GAT CCG GCT GTG GAA TGT GTG TCA GTT AGG GTG
 CCG TCG GTT CGA TCT CTA GGC CGA CAC CTT ACA CAC AGT CAA TCC CAC

       3320           3330           3340           3350           3360
        *             *              *              *              *
 TGG AAA GTC CCC AGG CTC CCC AGC AGG CAG AAG TAT GCA AAG CAT GCA
 ACC TTT CAG GGG TCC GAG GGG TCG TCC GTC TTC ATA CGT TTC GTA CGT

       3370           3380           3390           3400           3410
        *             *              *              *              *
 TCT CAA TTA GTC AGC AAC CAG GCT CCC CAG CAG GCA GAA GTA TGC AAA
 AGA GTT AAT CAG TCG TTG GTC CGA GGG GTC GTC CGT CTT CAT ACG TTT

          3420           3430           3440           3450
           *             *              *              *
 GCA TGC ATC TCA ATT AGT CAG CAA CCA TAG TCC CGC CCC TAA CTC CGC
 CGT ACG TAG AGT TAA TCA GTC GTT GGT ATC AGG GCG GGG ATT GAG GCG

    3460           3470           3480           3490           3500
     *             *              *              *              *
 CCA TCC CGC CCC TAA CTC CGC CCA GTT CCG CCC ATT CTC CGC CCC ATG
 GGT AGG GCG GGG ATT GAG GCG GGT CAA GGC GGG TAA GAG GCG GGG TAC

    3510           3520           3530           3540           3550
     *             *              *              *              *
 GCT GAC TAA TTT TTT TTA TTT ATG CAG AGG CCG AGG CCG CCT CGG CCT
 CGA CTG ATT AAA AAA AAT AAA TAC GTC TCC GGC TCC GGC GGA GCC GGA

       3560           3570           3580           3590           3600
        *             *              *              *              *
 CTG AGC TAT TCC AGA AGT AGT GAG GAG GCT TTT TTG GAG GCC TAG GCT
 GAC TCG ATA AGG TCT TCA TCA CTC CTC CGA AAA AAC CTC CGG ATC CGA

          3610           3620           3630           3640           3650
           *             *              *              *              *
 TTT GCA AAA AGC TAC CTT GGG GCC ACC GCT CAG AGC ACC TTC CAC CAT
 AAA CGT TTT TCG ATC GAA CCC CGG TGG CGA GTC TCG TGG AAG GTG GTA

          3660           3670           3680           3690
           *             *              *              *
 GGC CAC CTC AGC AAG TTC CCA CTT GAA CAA AAA CAT CAA GCA AAT GTA
 CCG GTG GAG TCG TTC AAG GGT GAA CTT GTT TTT GTA GTT CGT TTA CAT

    3700           3710           3720           3730           3740
     *             *              *              *              *
 CTT GTG CCT GCC CCA GGG TGA GAA AGT CCA AGC CAT GTA TAT CTG GGT
 GAA CAC GGA CGG GGT CCC ACT CTT TCA GGT TCG GTA CAT ATA GAC CCA

    3750           3760           3770           3780           3790
     *             *              *              *              *
 TGA TGG TAC TGG AGA AGG ACT GCG CTG CAA AAC CCG CAC CCT GGA CTG
 ACT ACC ATG ACC TCT TCC TGA CGC GAC GTT TTG GGC GTG GGA CCT GAC
```

FIG. 5 F

```
        2650            2660            2670            2680            2690
         •               •               •               •               •
ACC GAG TTG CTC TTG CCC GGC GTC AAC ACG GGA TAA TAC CGC GCC ACA
TGG CTC AAC GAG AAC GGG CCG CAG TTG TGC CCT ATT ATG GCG CGG TGT

         2700            2710            2720            2730
          •               •               •               •
TAG CAG AAC TTT AAA AGT GCT CAT CAT TGG AAA ACG TTC TTC GGG GCG
ATC GTC TTG AAA TTT TCA CGA GTA GTA ACC TTT TGC AAG AAG CCC CGC

    2740          2750            2760            2770            2780
     •             •               •               •               •
AAA ACT CTC AAG GAT CTT ACC GCT GTT GAG ATC CAG TTC GAT GTA ACC
TTT TGA GAG TTC CTA GAA TGG CGA CAA CTC TAG GTC AAG CTA CAT TGG

    2790          2800            2810            2820            2830
     •             •               •               •               •
CAC TCG TGC ACC CAA CTG ATC TTC AGC ATC TTT TAC TTT CAC CAG CGT
GTG AGC ACG TGG GTT GAC TAG AAG TCG TAG AAA ATG AAA GTG GTC GCA

        2840            2850            2860            2870            2880
         •               •               •               •               •
TTC TGG GTG AGC AAA AAC AGG AAG GCA AAA TGC CGC AAA AAA GGG AAT
AAG ACC CAC TCG TTT TTG TCC TTC CGT TTT ACG GCG TTT TTT CCC TTA

        2890           .2900           2910            2920            2930
         •               •               •               •               •
AAG GGC GAC ACG GAA ATG TTG AAT ACT CAT ACT CTT CCT TTT TCA ATA
TTC CCG CTG TGC CTT TAC AAC TTA TGA GTA TGA GAA GGA AAA AGT TAT

        2940            2950            2960            2970
         •               •               •               •
TTA TTG AAG CAT TTA TCA GGG TTA TTG TCT CAT GAG CCG ATA CAT ATT
AAT AAC TTC GTA AAT AGT CCC AAT AAC AGA GTA CTC GCC TAT GTA TAA

    2980          2990            3000            3010            3020
     •             •               •               •               •
TGA ATG TAT TTA GAA AAA TAA ACA AAT AGG GGT TCC GCG CAC ATT TCC
ACT TAC ATA AAT CTT TTT ATT TGT TTA TCC CCA AGG CGC GTG TAA AGG

    3030          3040            3050            3060            3070
     •             •               •               •               •
CCG AAA AGT GCC ACC TGA CGT CTA AGA AAC CAT TAT TAT CAT GAC ATT
GGC TTT TCA CGG TGG ACT GCA GAT TCT TTG GTA ATA ATA GTA CTG TAA

    3080          3090            3100            3110          - 3120
     •             •               •               •               •
AAC CTA TAA AAA TAG GCG TAT CAC GAG GCC CTG ATG GCT CTT TGC GGC
TTG GAT ATT TTT ATC CGC ATA GTG CTC CGG GAC TAC CGA GAA ACG CCG

        3130            3140            3150            3160            3170
         •               •               •               •               •
ACC CAT CGT TCG TAA TGT TCC GTG GCA CCG AGG ACA ACC CTC AAG AGA
TGG GTA GCA AGC ATT ACA AGG CAC CGT GGC TCC TGT TGG GAG TTC TCT

        3180            3190            3200            3210
         •               •               •               •
AAA TGT AAT CAC ACT GGC TCA CCT TCG GGT GGG CCT TTC TGC GTT TAT
TTT ACA TTA GTG TGA CCG AGT GGA AGC CCA CCC GGA AAG ACG CAA ATA
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FIG. 5 E

```
      2070            2080            2090            2100            2110
       •               •               •               •               •
    CAG TGA GGC ACC TAT CTC AGC GAT CTG TCT ATT TCG TTC ATC CAT AGT
    GTC ACT CCG TGG ATA GAG TCG CTA GAC AGA TAA AGC AAG TAG GTA TCA


          2120            2130            2140            2150            2160
           •               •               •               •               •
    TGC CTG ACT CCC CGT CGT GTA GAT AAC TAC GAT ACG GGA GGG CTT ACC
    ACG GAC TGA GGG GCA GCA CAT CTA TTG ATG CTA TGC CCT CCC GAA TGG


          2170            2180            2190            2200            2210
           •               •               •               •               •
    ATC TGG CCC CAG TGC TGC AAT GAT ACC GCG AGA CCC ACG CTC ACC GGC
    TAG ACC GGG GTC ACG ACG TTA CTA TGG CGC TCT GGG TGC GAG TGG CCG


             2220            2230            2240            2250
              •               •               •               •
    TCC AGA TTT ATC AGC AAT AAA CCA GCC AGC CGG AAG GGC CGA GCG CAG
    AGG TCT AAA TAG TCG TTA TTT GGT CGG TCG GCC TTC CCG GCT CGC GTC


    2260            2270            2280            2290            2300
     •               •               •               •               •
    AAG TGG TCC TGC AAC TTT ATC CGC CTC CAT CCA GTC TAT TAA TTG TTG
    TTC ACC AGG ACG TTG AAA TAG GCG GAG GTA GGT CAG ATA ATT AAC AAC


    2310            2320            2330            2340            2350
     •               •               •               •               •
    CCG GGA AGC TAG AGT AAG TAG TTC GCC AGT TAA TAG TTT GCG CAA CGT
    GGC CCT TCG ATC TCA TTC ATC AAG CGG TCA ATT ATC AAA CGC GTT GCA


       2360            2370            2380            2390            2400
        •               •               •               •               •
    TGT TGC CAT TGC TAC AGG CAT CGT GGT GTC ACG CTC GTC GTT TGG TAT
    ACA ACG GTA ACG ATG TCC GTA GCA CCA CAG TGC GAG CAG CAA ACC ATA


          2410            2420            2430            2440            2450
           •               •               •               •               •
    GGC TTC ATT CAG CTC CGG TTC CCA ACG ATC AAG GCG AGT TAC ATG ATC
    CCG AAG TAA GTC GAG GCC AAG GGT TGC TAG TTC CGC TCA ATG TAC TAG


          2460            2470            2480            2490
           •               •               •               •
    CCC CAT GTT GTG CAA AAA AGC GGT TAG CTC CTT CGG TCC TCC GAT CGT
    GGG GTA CAA CAC GTT TTT TCG CCA ATC GAG GAA GCC AGG AGG CTA GCA


    2500            2510            2520            2530            2540
     •               •               •               •               •
    TGT CAG AAG TAA GTT GGC CGC AGT GTT ATC ACT CAT GGT TAT GGC AGC
    ACA GTC TTC ATT CAA CCG GCG TCA CAA TAG TGA GTA CCA ATA CCG TCG


    2550            2560            2570            2580            2590
     •               •               •               •               •
    ACT GCA TAA TTC TCT TAC TGT CAT GCC ATC CGT AAG ATG CTT TTC TGT
    TGA CGT ATT AAG AGA ATG ACA GTA CGG TAG GCA TTC TAC GAA AAG ACA


       2600            2610            2620            2630            2640
        •               •               •               •               •
    GAC TGG TGA GTA CTC AAC CAA GTC ATT CTG AGA ATA GTG TAT GCG GCG
    CTG ACC ACT CAT GAG TTG GTT CAG TAA GAC TCT TAT CAC ATA CGC CGC
```

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FIG. 5 D

```
         1500            1510            1520            1530
          •               •               •               •
CTC ACG CTG TAG GTA TCT CAG TTC GGT GTA GGT CGT TCG CTC CAA GCT
GAG TGC GAC ATC CAT AGA GTC AAG CCA CAT CCA GCA AGC GAG GTT CGA

  1540            1550            1560            1570            1580
   •               •               •               •               •
GGG CTG TGT GCA CGA ACC CCC CGT TCA GCC CGA CCG CTG CGC CTT ATC
CCC GAC ACA CGT GCT TGG GGG GCA AGT CGG GCT GGC GAC GCG GAA TAG

   1590            1600            1610            1620            1630
    •               •               •               •               •
CGG TAA CTA TCG TCT TGA GTC CAA CCC GGT AAG ACA CGA CTT ATC GCC
GCC ATT GAT AGC AGA ACT CAG GTT GGG CCA TTC TGT GCT GAA TAG CGG

     1640            1650            1660            1670            1680
      •               •               •               •               •
ACT GGC AGC AGC CAC TGG TAA CAG GAT TAG CAG AGC GAG GTA TGT AGG
TGA CCG TCG TCG GTG ACC ATT GTC CTA ATC GTC TCG CTC CAT ACA TCC

        1690            1700            1710            1720            1730
         •               •               •               •               •
CGG TGC TAC AGA GTT CTT GAA GTG GTG GCC TAA CTA CGG CTA CAC TAG
GCC ACG ATG TCT CAA GAA CTT CAC CAC CGG ATT GAT GCC GAT GTG ATC

          1740            1750            1760            1770
           •               •               •               •
AAG GAC AGT ATT TCG TAT CTG CGC TCT GCT GAA GCC AGT TAC CTT CGG
TTC CTG TCA TAA ACC ATA GAC GCG AGA CGA CTT CGG TCA ATG GAA GCC

  1780            1790            1800            1810            1820
   •               •               •               •               •
AAA AAG AGT TGG TAG CTC TTG ATC CGG CAA ACA AAC CAC CGC TGG TAG
TTT TTC TCA ACC ATC GAG AAC TAG GCC GTT TGT TTG GTG GCG ACC ATC

 1830            1840            1850            1860            1870
  •               •               •               •               •
CGG TGG TTT TTT TGT TTG CAA GCA GCA GAT TAC GCG CAG AAA AAA AGG
GCC ACC AAA AAA ACA AAC GTT CGT CGT CTA ATG CGC GTC TTT TTT TCC

    1880            1890            1900            1910            1920
     •               •               •               •               •
ATC TCA AGA AGA TCC TTT GAT CTT TTC TAC GGG GTC TGA CGC TCA GTG
TAG AGT TCT TCT AGG AAA CTA GAA AAG ATG CCC CAG ACT GCG AGT CAC

      1930            1940            1950            1960            1970
       •               •               •               •               •
GAA CGA AAA CTC ACG TTA AGG GAT TTT GGT CAT GAG ATT ATC AAA AAG
CTT GCT TTT GAG TGC AAT TCC CTA AAA CCA GTA CTC TAA TAG TTT TTC

      1980            1990            2000            2010
       •               •               •               •
GAT CTT CAC CTA GAT CCT TTT AAA TTA AAA ATG AAG TTT TAA ATC AAT
CTA GAA GTG GAT CTA GGA AAA TTT AAT TTT TAC TTC AAA ATT TAG TTA

 2020            2030            2040            2050            2060
  •               •               •               •               •
CTA AAG TAT ATA TGA GTA AAC TTG GTC TGA CAG TTA CCA ATG CTT AAT
GAT TTC ATA TAT ACT CAT TTG AAC CAG ACT GTC AAT GGT TAC GAA TTA
```

FIG. 5 C

```
      920           930           940           950           960
       •             •             •             •             •
  TCA CAA ATA AAG CAT TTT TTT CAC TGC ATT CTA GTT GTG GTT TGT CCA
  AGT GTT TAT TTC GTA AAA AAA GTG ACG TAA GAT CAA CAC CAA ACA GGT

         970           980           990          1000          1010
          •             •             •             •             •
  AAC TCA TCA ATG TAT CTT ATC ATG TCT GGA TCC TCT ACG CCG GAC GCA
  TTG AGT AGT TAC ATA GAA TAG TAC AGA CCT AGG AGA TGC GGC CTG CGT

            1020          1030          1040          1050
             •             •             •             •
  TCG TGG CCG GCA TCA CCG GCG CCA CAG GTG CGG TTG CTG GCG CCT ATA
  AGC ACC GGC CGT AGT GGC CGC GGT GTC CAC GCC AAC GAC CGC GGA TAT

  1060          1070          1080          1090          1100
   •             •             •             •             •
  TCG CCG ACA TCA CCG ATG GGG AAG ATC GGG CTC GCC ACT TCG GGC TCA
  AGC GGC TGT AGT GGC TAC CCC TTC TAG CCC GAG CGG TGA AGC CCG AGT

  1110          1120          1130          1140          1150
   •             •             •             •             •
  TGA GCG CTT GTT TCG GCG TGG GTA TGG TGG CAG GCC CGT GGC CGG GGG
  ACT CGC GAA CAA AGC CGC ACC CAT ACC ACC GTC CGG GCA CCG GCC CCC

         1160          1170          1180          1190          1200
          •             •             •             •             •
  ACT GTT GGG CGC CAT CTC CTT GCA TGC ACC ATT CCT TGC GGC GGC GGT
  TGA CAA CCC GCG GTA GAG GAA CGT ACG TGG TAA GGA ACG CCG CCG CCA

            1210          1220          1230          1240          1250
             •             •             •             •             •
  GCT CAA CGG CCT CAA CCT ACT ACT GGG CTG CTT CCT AAT GCA GGA GTC
  CGA GTT GCC GGA GTT GGA TCA TGA CCC GAC GAA GGA TTA CGT CCT CAG

            1260          1270          1280          1290
             •             •             •             •
  GCA TAA GGG AGA GCG TCG ACC TCG GGC CGC GTT GCT GGC GTT TTT CCA
  CGT ATT CCC TCT CGC AGC TGG AGC CCG GCG CAA CGA CCG CAA AAA GGT

  1300          1310          1320          1330          1340
   •             •             •             •             •
  TAG GCT CCG CCC CCC TGA CGA GCA TCA CAA AAA TCG ACG CTC AAG TCA
  ATC CGA GGC GGG GGG ACT GCT CGT AGT GTT TTT AGC TGC GAG TTC AGT

  1350          1360          1370          1380          1390
   •             •             •             •             •
  GAG GTG GCG AAA CCC GAC AGG ACT ATA AAG ATA CCA GGC GTT TCC CCC
  CTC CAC CGC TTT GGG CTG TCC TGA TAT TTC TAT GGT CCG CAA AGG GGG

     1400          1410          1420          1430          1440
      •             •             •             •             •
  TGG AAG CTC CCT CGT GCG CTC TCC TGT TCC GAC CCT GCC GCT TAC CGG
  ACC TTC GAG GGA GCA CGC GAG AGG ACA AGG CTG GGA CGG CGA ATG GCC

        1450          1460          1470          1480          1490
         •             •             •             •             •
  ATA CCT GTC CGC CTT TCT CCC TTC GGG AAG CGT GGC GCT TTC TCA ATG
  TAT GGA CAG GCG GAA AGA GGG AAG CCC TTC GCA CCG CGA AAG AGT TAC
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FIG. 5 B

```
          440           450           460           470           480
           •             •             •             •             •
    GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC
    CTC GTC AAC TTT AGA CCT TGA CGG AGA CAA CAC ACG GAC GAC TTA TTG
    Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn>

              490           500           510           520           530
               •             •             •             •             •
    TTC TAT CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC
    AAG ATA GGG TCT CTC CGG TTT CAT GTC ACC TTC CAC CTA TTG CGG GAG
    Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu>

                 540           550           560           570
                  •             •             •             •
    CAA TCG GGT AAC TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC
    GTT AGC CCA TTG AGG GTC CTC TCA CAG TGT CTC GTC CTG TCG TTC CTG
    Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp>

    580           590           600           610           620
     •             •             •             •             •
    AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC
    TCG TGG ATG TCG GAG TCG TCG TGG GAC TGC GAC TCG TTT CGT CTG ATG
    Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr>

       630           640           650           660           670
        •             •             •             •             •
    GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC
    CTC TTT GTG TTT CAG ATG CGG ACG CTT CAG TGG GTA GTC CCG GAC TCG
    Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser>

          680           690           700           710           720
           •             •             •             •             •
    TCG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT T AGA GGG AGA AGT
    AGC GGG CAG TGT TTC TCG AAG TTG TCC CCT CTC ACA A TCT CCC TCT TCA
    Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys>

             730           740           750           760           770
              •             •             •             •             •
    GCC CCC ACC TGC TCC TCA GTT CCA GCC TGG GGA TCA TAA TCA GCC ATA
    CGG GGG TGG ACG AGG AGT CAA GGT CGG ACC CCT AGT ATT AGT CGG TAT

                780           790           800           810
                 •             •             •             •
    CCA CAT TTC TAG AGG TTT TAC TTG CTT TAA AAA ACC TCC CAC ACC TCC
    GGT GTA AAC ATC TCC AAA ATG AAC GAA ATT TTT TGG AGG GTG TGG AGG

    820           830           840           850           860
     •             •             •             •             •
    CCC TGA ACC TGA AAC ATA AAA TGA ATG CAA TTG TTG TTG TTA ACT TGT
    GGG ACT TGG ACT TTG TAT TTT ACT TAC GTT AAC AAC AAC AAT TGA ACA

    870           880           890           900           910
     •             •             •             •             •
    TTA TTG CAG CTT ATA ATG GTT ACA AAT AAA GCA ATA GCA TCA CAA ATT
    AAT AAC GTC GAA TAT TAC CAA TGT TTA TTT CGT TAT CGT AGT GTT TAA
```

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FIG. 5 A

The pEe12TF8LCDR3 expression vector DNA sequence. The coding regions of the TF8-5G9 CDR-grafted LC gene, TF8LCDR3, are translated.

Sequence Range: 1 to 7864

```
              10              20              30            40            50
              •               •               •             •             •
AAT TCA CC ATG GGT GTG CCA ACT CAG GTA TTA GGA TTA CTG CTG CTG TGG
TTA AGT GG TAC CCA CAC GGT TGA GTC CAT AAT CCT AAT GAC GAC GAC ACC
           Met Gly Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp>

              60              70              80            90
              •               •               •             •
CTT ACA GAT GCA AGA TGT GAT ATC CAA ATG ACA CAA TCT CCT TCT TCT
GAA TGT CTA CGT TCT ACA CTA TAG GTT TAC TGT GTT AGA GGA AGA AGA
Leu Thr Asp Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser>

100             110             120             130           140
  •               •               •               •             •
CTA AGT GCT TCT GTC GGA GAT AGA GTA ACA ATT ACA TGT AAG GCG AGT
GAT TCA CGA AGA CAG CCT CTA TCT CAT TGT TAA TGT ACA TTC CGC TCA
Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser>

      150             160             170             180           190
        •               •               •               •             •
CAG GAC ATT AGA AAG TAT TTA AAC TGG TAT CAG CAA AAA CCT GGG AAG
GTC CTG TAA TCT TTC ATA AAT TTG ACC ATA GTC GTT TTT GGA CCC TTC
Gln Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys>

        200             210             220             230           240
          •               •               •               •             •
GCT CCT AAG CTA CTG ATT TAT TAT GCA ACA AGT TTG GCA GAT GGA GTA
CGA GGA TTC GAT GAC TAA ATA ATA CGT TGT TCA AAC CGT CTA CCT CAT
Ala Pro Lys Leu Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val>

        250             260             270             280           290
          •               •               •               •             •
CCT TCT AGA TTT TCT GGT TCT GGC TCT GGA ACA GAC TAC ACA TTC ACA
GGA AGA TCT AAA AGA CCA AGA CCG AGA CCT TGT CTG ATG TGT AAG TGT
Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr>

        300             310             320             330
          •               •               •               •
ATT TCT TCT CTC CAA CCT GAG GAC ATT GCT ACA TAC TAC TGC CTA CAA
TAA AGA AGA GAG GTT GGA CTC CTG TAA CGA TGT ATG ATG ACG GAT GTT
Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln>

340             350             360             370           380
  •               •               •               •             •
CAT GGT GAG AGT CCG TAT ACA TTT GGA CAA GGA ACA AAA CTA GAG ATC
GTA CCA CTC TCA GGC ATA TGT AAA CCT GTT CCT TGT TTT GAT CTC TAG
His Gly Glu Ser Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile>

      390             400             410             420           430
        •               •               •               •             •
ACA AGA ACT GTT GCG GCG CCG TCT GTC TTC ATC TTC CCG CCA TCT GAT
TGT TCT TGA CAA CGC CGC GGC AGA CAG AAG TAG AAG GGC GGT AGA CTA
Thr Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp>
```

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FIG. 4 N

```
       6680          6690           6700          6710          6720
        •             •              •             •             •
      TGG AGG CCA GAC TTA GGC ACA GCA CGA TGC CCA CCA CCA CCA GTG TGC
      ACC TCC GGT CTG AAT CCG TGT CGT GCT ACG GGT GGT GGT GGT CAC ACG

         6730          6740          6750          6760          6770
          •             •             •             •             •
      CGC ACA AGG CCG TGG CGG TAG GGT ATG TGT CTG AAA ATG AGC TCG GGG
      GCG TGT TCC GGC ACC GCC ATC CCA TAC ACA GAC TTT TAC TCG AGC CCC

           6780          6790          6800          6810          6820
            •             •             •             •             •
      AGC GGG CTT GCA CCG CTG ACG CAT TTG GAA GAC TTA AGG CAG CGG CAG
      TCG CCC GAA CGT GGC GAC TGC GTA AAC CTT CTG AAT TCC GTC GCC GTC

             6830          6840          6850          6860          6870
              •             •             •             •             •
      AAG AAG ATC CAG GCA GCT GAG TTG TTG TGT TCT GAT AAG AGT CAG AGG
      TTC TTC TAC GTC CGT CGA CTC AAC AAC ACA AGA CTA TTC TCA GTC TCC

                6880          6890          6900          6910
                 •             •             •             •
      TAA CTC CCG TTG CGG TCC TGT TAA CCG TGG AGG GCA GTG TAG TCT GAG
      ATT GAG GGC AAC GCC ACG ACA ATT GCC ACC TCC CGT CAC ATC AGA CTC

      6920          6930          6940          6950          6960
       •             •             •             •             •
      CAG TAC TCG TTG CTG CCG CGC GCG CCA CCA GAC ATA ATA GCT GAC AGA
      GTC ATG AGC AAC GAC GGC GCG CGC GGT GGT CTG TAT TAT CGA CTG TCT

         6970          6980          6990          7000          7010
          •             •             •             •             •
      CTA ACA GAC TGT TCC TTT CCA TGG GTC TTT TCT GCA GTC ACC GTC CTT
      GAT TGT CTG ACA AGG AAA GGT ACC CAG AAA AGA CGT CAG TGG CAG GAA

            7020          7030          7040          7050          7060
             •             •             •             •             •
      GAC ACG AAG CTT GGG CTC CAG GTC GAT CGA CTC TAG AGG ATC GAT CCC
      CTG TGC TTC GAA CCC GAC GTC CAG CTA GCT GAG ATC TCC TAG CTA GGG

               7070
                •
      CGG GCG AGC TC
      GCC CGC TCG AG
```

FIG. 4 M

```
        6160            6170            6180            6190
          •               •               •               •
CGC GGA TTC CCC GTG CCA AGA GTG ACG TAA GTA CCG CCT ATA GAG TCT
GCG CCT AAG GGG CAC GGT TCT CAC TGC ATT CAT GGC GGA TAT CTC AGA

    6200            6210            6220            6230            6240
      •               •               •               •               •
ATA GGC CCA CCC CCT TGG CTT CTT ATG CAT GCT ATA CTG TTT TTG GCT
TAT CCG GGT GGG GGA ACC GAA GAA TAC GTA CGA TAT GAC AAA AAC CGA

        6250            6260            6270            6280            6290
          •               •               •               •               •
TGG GGT CTA TAC ACC CCC GCT TCC TCA TGT TAT AGG TGA TGG TAT AGC
ACC CCA GAT ATG TGG GGG CGA AGG AGT ACA ATA TCC ACT ACC ATA TCG

        6300            6310            6320            6330            6340
          •               •               •               •               •
TTA GCC TAT AGG TGT GGG TTA TTG ACC ATT ATT GAC CAC TCC CCT ATT
AAT CGG ATA TCC ACA CCC AAT AAC TGG TAA TAA CTG GTG AGG GGA TAA

        6350            6360            6370            6380            6390
          •               •               •               •               •
GGT GAC GAT ACT TTC CAT TAC TAA TCC ATA ACA TGG CTC TTT GCC ACA
CCA CTG CTA TGA AAG GTA ATG ATT AGG TAT TGT ACC GAG AAA CGG TGT

        6400            6410            6420            6430
          •               •               •               •
ACT CTC TTT ATT GGC TAT ATG CCA ATA CAC TGT CCT TCA GAG ACT GAC
TGA GAG AAA TAA CCG ATA TAC GGT TAT GTG ACA GGA AGT CTC TGA CTG

6440            6450            6460            6470            6480
  •               •               •               •               •
ACG GAC TCT GTA TTT TTA CAG GAT GGG GTC TCA TTT ATT ATT TAC AAA
TGC CTG AGA CAT AAA AAT GTC CTA CCC CAG AGT AAA TAA TAA ATG TTT

    6490            6500            6510            6520            6530
      •               •               •               •               •
TTC ACA TAT ACA ACA CCA CCG TCC CCA GTG CCC GCA GTT TTT ATT AAA
AAG TGT ATA TGT TGT GGT GGC AGG GGT CAC GGG CGT CAA AAA TAA TTT

    6540            6550            6560            6570            6580
      •               •               •               •               •
CAT AAC GTG GGA TCT CCA CGC GAA TCT CGG GTA CGT GTT CCG GAC ATG
GTA TTG CAC CCT AGA GGT GCG CTT AGA GCC CAT GCA CAA GGC CTG TAC

        6590            6600            6610            6620    -       6630
          •               •               •               •               •
GGC TCT TCT CCG GTA GCG GCG GAG CTT CTA CAT CCG AGC CCT GCT CCC
CCG AGA AGA GGC CAT CGC CGC CTC GAA GAT GTA GGC TCG GGA CGA GGG

        6640            6650            6660            6670
          •               •               •               •
ATG CCT CCA GCG ACT CAT GGT CGC TCG GCA GCT CCT TGC TCC TAA CAG
TAC GGA GGT CGC TGA GTA CCA GCG AGC CGT CGA GGA ACG AGG ATT GTC
```

FIG. 4 L

```
        5630            5640            5650            5660            5670
         •               •               •               •               •
TAA CGC CAA TAG GGA CTT TCC ATT GAC GTC AAT GGG TGG AGT ATT TAC
ATT GCG GTT ATC CCT GAA AGG TAA CTG CAG TTA CCC ACC TCA TAA ATG


                5680            5690            5700            5710
                 •               •               •               •
GGT AAA CTG CCC ACT TGG CAG TAC ATC AAG TGT ATC ATA TGC CAA GTA
CCA TTT GAC GGG TGA ACC GTC ATG TAG TTC ACA TAG TAT ACG GTT CAT


 5720            5730            5740            5750            5760
  •               •               •               •               •
CGC CCC CTA TTG ACG TCA ATG ACG GTA AAT GGC CCG CCT GGC ATT ATG
GCG GGG GAT AAC TGC AGT TAC TGC CAT TTA CCG GGC GGA CCG TAA TAC


        5770            5780            5790            5800            5810
         •               •               •               •               •
CCC AGT ACA TGA CCT TAT GGG ACT TTC CTA CTT GGC AGT ACA TCT ACG
GGG TCA TGT ACT GGA ATA CCC TGA AAG GAT GAA CCG TCA TGT AGA TGC


                5820            5830            5840            5850            5860
                 •               •               •               •               •
TAT TAG TCA TCG CTA TTA CCA TGG TGA TGC GGT TTT GGC AGT ACA TCA
ATA ATC AGT AGC GAT AAT GGT ACC ACT ACG CCA AAA CCG TCA TGT AGT


                5870            5880            5890            5900            5910
                 •               •               •               •               •
ATG GGC GTG GAT AGC GGT TTG ACT CAC GGG GAT TTC CAA GTC TCC ACC
TAC CCG CAC CTA TCG CCA AAC TGA GTG CCC CTA AAG GTT CAG AGG TGG


                5920            5930            5940            5950
                 •               •               •               •
CCA TTG ACG TCA ATG GGA GTT TGT TTT GGC ACC AAA ATC AAC GGG ACT
GGT AAC TGC AGT TAC CCT CAA ACA AAA CCG TGG TTT TAG TTG CCC TGA


 5960            5970            5980            5990            6000
  •               •               •               •               •
TTC CAA AAT GTC GTA ACA ACT CCG CCC CAT TGA CGC AAA TGG GCG GTA
AAG GTT TTA CAG CAT TGT TGA GGC GGG GTA ACT GCG TTT ACC CGC CAT


 6010            6020            6030            6040            6050
  •               •               •               •               •
GGC GTG TAC GGT GGG AGG TCT ATA TAA GCA GAG CTC GTT TAG TGA ACC
CCG CAC ATG CCA CCC TCC AGA TAT ATT CGT CTC GAG CAA ATC ACT TGG


        6060            6070            6080            6090            6100
         •               •               •               •               •
GTC AGA TCG CCT GGA GAC GCC ATC CAC GCT GTT TTG ACC TCC ATA GAA
CAG TCT AGC GGA CCT CTG CGG TAG GTG CGA CAA AAC TGG AGG TAT CTT


                6110            6120            6130            6140            6150
                 •               •               •               •               •
GAC ACC GGG ACC GAT CCA GCC TCC GCG GCC GGG AAC GGT GCA TTG GAA
CTG TGG CCC TGG CTA GGT CGG AGG CGC CGG CCC TTG CCA CGT AAC CTT
```

FIG. 4 K

```
     5100            5110            5120            5130            5140
       •               •               •               •               •
ATA TCG CCA TTT TTC CAA AAG TGA TTT TTG GGC ATA CGC GAT ATC TGG
TAT AGC GGT AAA AAG GTT TTC ACT AAA AAC CCG TAT GCG CTA TAG ACC

         5150            5160            5170            5180            5190
           •               •               •               •               •
CGA TAG CGC TTA TAT CGT TTA CGG GGG ATG GCG ATA GAC GAC TTT GGT
GCT ATC GCG AAT ATA GCA AAT GCC CCC TAC CGC TAT CTG CTG AAA CCA

           5200            5210            5220            5230
             •               •               •               •
GAC TTG GGC GAT TCT GTG TGT CGC AAA TAT CGC AGT TTC GAT ATA GGT
CTG AAC CCG CTA AGA CAC ACA GCG TTT ATA GCG TCA AAG CTA TAT CCA

5240            5250            5260            5270            5280
  •               •               •               •               •
GAC AGA CGA TAT GAG GCT ATA TCG CCG ATA GAG GCG ACA TCA AGC TGG
CTG TCT GCT ATA CTC CGA TAT AGC GGC TAT CTC CGC TGT AGT TCG ACC

     5290            5300            5310            5320            5330
       •               •               •               •               •
CAC ATG GCC AAT GCA TAT CGA TCT ATA CAT TGA ATC AAT ATT GGC CAT
GTG TAC CGG TTA CGT ATA GCT AGA TAT GTA ACT TAG TTA TAA CCG GTA

       5340            5350            5360            5370            5380
         •               •               •               •               •
TAG CCA TAT TAT TCA TTG GTT ATA TAG CAT AAA TCA ATA TTG GCT ATT
ATC GGT ATA ATA AGT AAC CAA TAT ATC GTA TTT AGT TAT AAC CGA TAA

         5390            5400            5410            5420            5430
           •               •               •               •               •
GGC CAT TGC ATA CGT TGT ATC CAT ATC ATA ATA TGT ACA TTT ATA TTG
CCG GTA ACG TAT GCA ACA TAG GTA TAG TAT TAT ACA TGT AAA TAT AAC

           5440            5450            5460            5470
             •               •               •               •
GCT CAT GTC CAA CAT TAC CGC CAT GTT GAC ATT GAT TAT TGA CTA GTT
CGA GTA CAG GTT GTA ATG GCG GTA CAA CTG TAA CTA ATA ACT GAT CAA

5480            5490            5500            5510            5520
  •               •               •               •               •
ATT AAT AGT AAT CAA TTA CGG GGT CAT TAG TTC ATA GCC CAT ATA TGG
TAA TTA TCA TTA GTT AAT GCC CCA GTA ATC AAG TAT CGG GTA TAT ACC

     5530            5540            5550            5560            5570
       •               •               •               •               •
AGT TCC GCG TTA CAT AAC TTA CGG TAA ATG GCC CGC CTG GCT GAC CGC
TCA AGG CGC AAT GTA TTG AAT GCC ATT TAC CGG GCG GAC CGA CTG GCG

       5580            5590            5600            5610            5620
         •               •               •               •               •
CCA ACG ACC CCC GCC CAT TGA CGT CAA TAA TGA CGT ATG TTC CCA TAG
GGT TGC TGG GGG CGG GTA ACT GCA GTT ATT ACT GCA TAC AAG GGT ATC
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FIG. 4 J

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      4570         4580          4590          4600         4610
       •            •             •             •            •
AGC CGA TAC ATA TTT GAA TGT ATT TAG AAA AAT AAA CAA ATA GGG GTT
TCG CCT ATG TAT AAA CTT ACA TAA ATC TTT TTA TTT GTT TAT CCC CAA

         4620         4630          4640          4650         4660
          •            •             •             •            •
CCG CGC ACA TTT CCC CGA AAA GTG CCA CCT GAC GTC TAA GAA ACC ATT
GGC GCG TGT AAA GGG GCT TTT CAC GGT GGA CTG CAG ATT CTT TGG TAA

            4670         4680          4690          4700         4710
             •            •             •             •            •
ATT ATC ATG ACA TTA ACC TAT AAA AAT AGG CGT ATC ACG AGG CCC TGA
TAA TAG TAC TGT AAT TGG ATA TTT TTA TCC GCA TAG TGC TCC GGG ACT

               4720         4730          4740          4750
                •            •             •             •
TGG CTC TTT GCG GCA CCC ATC GTT CGT AAT GTT CCG TGG CAC CGA GGA
ACC GAG AAA CGC CGT GGG TAG CAA GCA TTA CAA GGC ACC GTG GCT CCT

4760         4770          4780          4790          4800
 •            •             •             •             •
CAA CCC TCA AGA CAA AAT GTA ATC ACA CTG GCT CAC CTT CGG GTG GGC
GTT GGG AGT TCT CTT TTA CAT TAG TGT GAC CGA GTG GAA GCC CAC CCG

      4810         4820          4830          4840         4850
       •            •             •             •            •
CTT TCT GCG TTT ATA AGG AGA CAC TTT ATG TTT AAG AAG GTT GGT AAA
GAA AGA CGC AAA TAT TCC TCT GTG AAA TAC AAA TTC TTC CAA CCA TTT

         4860         4870          4880          4890         4900
          •            •             •             •            •
TTC CTT GCG GCT TTG GCA GCC AAG CTA GAG ATC TCT AGC TTC GTG TCA
AAG GAA CGC CGA AAC CGT CGG TTC GAT CTC TAG AGA TCG AAG CAC AGT

            4910         4920          4930          4940         4950
             •            •             •             •            •
AGG ACG GTG ACT GCA GTG AAT AAT AAA ATG TGT GTT TGT CCG AAA TAC
TCC TGC CAC TGA CGT CAC TTA TTA TTT TAC ACA CAA ACA GGC TTT ATG

               4960         4970          4980          4990
                •            •             •             •
GCG TTT TGA GAT TTC TGT CGC CGA CTA AAT TCA TGT CGC GCG ATA GTG
CGC AAA ACT CTA AAG ACA GCG GCT GAT TTA AGT ACA GCG CGC TAT CAC

5000         5010          5020          5030          5040
 •            •             •             •             •
GTG TTT ATC GCC GAT AGA GAT GGC GAT ATT GGA AAA ATC GAT ATT TGA
CAC AAA TAG CGG CTA TCT CTA CCG CTA TAA CCT TTT TAG CTA TAA ACT

      5050         5060          5070          5080         5090
       •            •             •             •            •
AAA TAT GGC ATA TTG AAA ATG TCG CCG ATG TGA GTT TCT GTC TAA CTG
TTT ATA CCG TAT AAC TTT TAC AGC GGC TAC ACT CAA AGA CAC ATT GAC
```

FIG. 4 I

```
     4040          4050          4060          4070          4080
      •             •             •             •             •
CGA GTT ACA TGA TCC CCC ATG TTG TGC AAA AAA GCC GTT AGC TCC TTC
GCT CAA TGT ACT AGG GGG TAC AAC ACG TTT TTT CGC CAA TCG AGG AAG

         4090          4100          4110          4120       4130
          •             •             •             •           •
GGT CCT CCG ATC GTT GTC AGA AGT AAG TTG CCC GCA GTG TTA TCA CTC
CCA GGA GGC TAG CAA CAG TCT TCA TTC AAC CGG CGT CAC AAT AGT GAG

         4140          4150          4160          4170          4180
          •             •             •             •             •
ATG GTT ATG GCA GCA CTC CAT AAT TCT CTT ACT GTC ATG CCA TCC GTA
TAC CAA TAC CGT CGT GAC GTA TTA AGA GAA TGA CAG TAC GGT AGG CAT

         4190          4200          4210          4220          4230
          •             •             •             •             •
AGA TGC TTT TCT GTG ACT GGT GAG TAC TCA ACC AAG TCA TTC TGA GAA
TCT ACG AAA AGA CAC TGA CCA CTC ATG AGT TGG TTC AGT AAG ACT CTT

         4240          4250          4260          4270
          •             •             •             •
TAG TGT ATG CGG CGA CCG AGT TGC TCT TGC CCG GCG TCA ACA CGG GAT
ATC ACA TAC GCC GCT GGC TCA ACG AGA ACG GGC CGC AGT TGT GCC CTA

    4280          4290          4300          4310          4320
     •             •             •             •             •
AAT ACC GCG CCA CAT AGC AGA ACT TTA AAA GTG CTC ATC ATT GGA AAA
TTA TGG CGC GGT GTA TCG TCT TGA AAT TTT CAC GAG TAG TAA CCT TTT

    4330          4340          4350          4360          4370
     •             •             •             •             •
CGT TCT TCG GGG CGA AAA CTC TCA AGG ATC TTA CCG CTG TTG AGA TCC
GCA AGA AGC CCC GCT TTT GAG AGT TCC TAG AAT GGC GAC AAC TCT AGG

    4380          4390          4400          4410          4420
     •             •             •             •             •
AGT TCG ATG TAA CCC ACT CGT GCA CCC AAC TGA TCT TCA GCA TCT TTT
TCA AGC TAC ATT GGG TGA GCA CGT GGG TTG ACT AGA AGT CGT AGA AAA

         4430          4440          4450          4460          4470
          •             •             •             •             •
ACT TTC ACC AGC GTT TCT GGG TGA GCA AAA ACA GGA AGG CAA AAT CCC
TGA AAG TGG TCG CAA AGA CCC ACT CGT TTT TGT CCT TCC GTT TTA GGG

         4480          4490          4500          4510
          •             •             •             •
GCA AAA AAG GGA ATA AGG GCG ACA CGG AAA TGT TGA ATA CTC ATA CTC
CGT TTT TTC CCT TAT TCC CGC TGT GCC TTT ACA ACT TAT GAG TAT GAG

    4520          4530          4540          4550          4560
     •             •             •             •             •
TTC CTT TTT CAA TAT TAT TGA AGC ATT TAT CAG GGT TAT TGT CTC ATG
AAG GAA AAA GTT ATA ATA ACT TCG TAA ATA GTC CCA ATA ACA GAG TAC
```

FIG. 4 H

```
           3520            3530            3540            3550
            •               •               •               •
      TCT GAC GCT CAG TGG AAC GAA AAC TCA CGT TAA GGG ATT TTG GTC ATG
      AGA CTG CGA GTC ACC TTG CTT TTG AGT GCA ATT CCC TAA AAC CAG TAC

     3560        3570            3580            3590         3600
      •           •               •               •           •
      AGA TTA TCA AAA AGG ATC TTC ACC TAG ATC CTT TTA AAT TAA AAA TGA
      TCT AAT AGT TTT TCC TAG AAG TGG ATC TAG GAA AAT TTA ATT TTT ACT

         3610            3620            3630            3640        3650
          •               •               •               •           •
      AGT TTT AAA TCA ATC TAA AGT ATA TAT GAG TAA ACT TGG TCT GAC AGT
      TCA AAA TTT AGT TAG ATT TCA TAT ATA CTC ATT TGA ACC AGA CTG TCA

           3660            3670            3680            3690        3700
            •               •               •               •           •
      TAC CAA TGC TTA ATC AGT GAG GCA CCT ATC TCA GCG ATC TGT CTA TTT
      ATG GTT ACG AAT TAG TCA CTC CGT GGA TAG AGT CGC TAG ACA GAT AAA

           3710            3720            3730            3740        3750
            •               •               •               •           •
      CGT TCA TCC ATA GTT GCC TGA CTC CCC GTC GTG TAG ATA ACT ACG ATA
      GCA AGT AGG TAT CAA CGG ACT GAG GGG CAG CAC ATC TAT TGA TGC TAT

              3760            3770            3780            3790
               •               •               •               •
      CGG GAG GGC TTA CCA TCT GGC CCC AGT GCT GCA ATG ATA CCG CGA GAC
      GCC CTC CCG AAT GGT AGA CCG GGG TCA CGA CGT TAC TAT GGC GCT CTG

     3800        3810            3820            3830        3840
      •           •               •               •           •
      CCA CGC TCA CCG GCT CCA GAT TTA TCA GCA ATA AAC CAG CCA GCC GGA
      GGT GCG AGT GGC CGA GGT CTA AAT AGT CGT TAT TTG GTC GGT CGG CCT

         3850            3860            3870            3880        3890
          •               •               •               •           •
      AGG GCC GAG CGC AGA AGT GGT CCT GCA ACT TTA TCC GCC TCC ATC CAG
      TCC CGG CTC GCG TCT TCA CCA GGA CGT TGA AAT AGG CGG AGG TAG GTC

         3900            3910            3920            3930        3940
          •               •               •               •           •
      TCT ATT AAT TGT TGC CGG GAA GCT AGA GTA AGT AGT TCG CCA GTT AAT
      AGA TAA TTA ACA ACG GCC CTT CGA TCT CAT TCA TCA AGC GGT CAA TTA

         3950            3960            3970            3980        3990
          •               •               •               •           •
      AGT TTG CGC AAC GTT GTT GCC ATT GCT ACA GGC ATC GTG GTG TCA CGC
      TCA AAC GCG TTG CAA CAA CGG TAA CGA TGT CCG TAG CAC CAC AGT GCG

            4000            4010            4020            4030
             •               •               •               •
      TCG TCG TTT GGT ATG GCT TCA TTC AGC TCC GGT TCC CAA CGA TCA AGG
      AGC AGC AAA CCA TAC CGA AGT AAG TCG AGG CCA AGG GTT GCT AGT TCC
```

FIG. 4 G

```
      2990            3000            3010            3020            3030
       •               •               •               •               •
CAG GCG TTT CCC CCT GGA AGC TCC CTC GTG CGC TCT CCT GTT CCC ACC
GTC CGC AAA GGG GGA CCT TCG AGG GAG CAC GCG AGA GGA CAA GGC TGG


           3040            3050            3060            3070
            •               •               •               •
CTG CCG CTT ACC GGA TAC CTG TCC GCC TTT CTC CCT TCG GGA AGC GTG
GAC GGC GAA TGG CCT ATG GAC AGG CGG AAA GAG GGA AGC CCT TCG CAC


  3080            3090            3100            3110            3120
   •               •               •               •               •
GCG CTT TCT CAA TGC TCA CGC TGT AGG TAT CTC AGT TCG GTG TAG GTC
CGC GAA AGA GTT ACG AGT GCG ACA TCC ATA GAG TCA AGC CAC ATC CAG


     3130            3140            3150            3160            3170
      •               •               •               •               •
GTT CGC TCC AAG CTG GGC TGT GTG CAC GAA CCC CCC GTT CAG CCC GAC
CAA GCG AGG TTC GAC CCG ACA CAC GTG CTT GGG GGG CAA GTC GGG CTC


       3180            3190            3200            3210            3220
        •               •               •               •               •
CGC TGC GCC TTA TCC GGT AAC TAT CGT CTT GAG TCC AAC CCG GTA AGA
GCG ACG CGG AAT AGG CCA TTG ATA GCA GAA CTC AGG TTG GGC CAT TCT


         3230            3240            3250            3260            3270
          •               •               •               •               •
CAC GAC TTA TCG CCA CTG GCA GCA GCC ACT GGT AAC AGG ATT AGC AGA
GTG CTG AAT AGC GGT GAC CGT CGT CGG TGA CCA TTG TCC TAA TCG TCT


           3280            3290            3300            3310
            •               •               •               •
GCG AGG TAT GTA GGC GGT GCT ACA GAG TTC TTG AAG TGG TGG CCT AAC
CGC TCC ATA CAT CCG CCA CGA TGT CTC AAG AAC TTC ACC ACC GGA TTG


3320            3330            3340            3350            3360
 •               •               •               •               •
TAC GGC TAC ACT AGA AGG ACA GTA TTT GGT ATC TGC GCT CTC CTG AAG
ATG CCG ATG TGA TCT TCC TGT CAT AAA CCA TAG ACG CGA GAC GAC TTC


      3370            3380            3390            3400            3410
       •               •               •               •               •
CCA GTT ACC TTC GGA AAA AGA GTT GGT AGC TCT TGA TCC GGC AAA CAA
GGT CAA TGG AAG CCT TTT TCT CAA CCA TCG AGA ACT AGG CCG TTT GTT


      3420            3430            3440            3450            3460
       •               •               •               •               •
ACC ACC GCT GGT AGC GGT GGT TTT TTT GTT TGC AAG CAG CAG ATT ACG
TGG TGG CGA CCA TCG CCA CCA AAA AAA CAA ACG TTC GTC GTC TAA TGC


        3470            3480            3490            3500            3510
         •               •               •               •               •
CGC AGA AAA AAA GGA TCT CAA GAA GAT CCT TTG ATC TTT TCT ACG GGG
GCG TCT TTT TTT CCT AGA GTT CTT CTA GGA AAC TAG AAA AGA TGC CCC
```

FIG. 4 F

```
        2460            2470            2480            2490            2500
         •               •               •               •               •
TGT TGT TAA CTT GTT TAT TGC AGC TTA TAA TGG TTA CAA ATA AAG CAA
ACA ACA ATT GAA CAA ATA ACG TCG AAT ATT ACC AAT GTT TAT TTC GTT

           2510            2520            2530            2540            2550
            •               •               •               •               •
TAG CAT CAC AAA TTT CAC AAA TAA AGC ATT TTT TTC ACT GCA TTC TAG
ATC GTA GTG TTT AAA GTG TTT ATT TCG TAA AAA AAG TGA CGT AAG ATC

             2560            2570            2580            2590
              •               •               •               •
TTG TGG TTT GTC CAA ACT CAT CAA TGT ATC TTA TCA TGT CTG GAT CCT
AAC ACC AAA CAG GTT TGA GTA GTT ACA TAG AAT AGT ACA GAC CTA GGA

    2600            2610            2620            2630            2640
     •               •               •               •               •
CTA CGC CGG ACG CAT CGT GGC CGG CAT CAC CGG CGC CAC AGG TGC GGT
GAT GCG GCC TGC GTA GCA CCG GCC GTA GTG GCC GCG GTG TCC ACG CCA

           2650            2660            2670            2680            2690
            •               •               •               •               •
TGC TGG CGC CTA TAT CGC CGA CAT CAC CGA TGG GGA AGA TCG GGC TCG
ACG ACC GCG GAT ATA GCG GCT GTA GTG GCT ACC CCT TCT AGC CCG AGC

        2700            2710            2720            2730            2740
         •               •               •               •               •
CCA CTT CGG CCT CAT GAG CGC TTG TTT CGG CGT GGG TAT GGT GGC AGG
GGT GAA GCC CGA GTA CTC GCG AAC AAA GCC GCA CCC ATA CCA CCG TCC

           2750            2760            2770            2780            2790
            •               •               •               •               •
CCC GTG GCC GGG GGA CTG TTG GGC GCC ATC TCC TTG CAT GCA CCA TTC
GGG CAC CGG CCC CCT GAC AAC CCG CGG TAG AGG AAC GTA CGT CGT AAG

              2800            2810            2820            2830
               •               •               •               •
CTT GCG GCG GCG GTG CTC AAC GGC CTC AAC CTA CTA CTG GGC TGC TTC
GAA CGC CGC CGC CAC GAG TTG CCG GAG TTG GAT GAT GAC CCG ACG AAG

    2840            2850            2860            2870            2880
     •               •               •               •               •
CTA ATG CAG GAG TCG CAT AAG GGA GAG CGT CGA CCT CGG GCC GCG TTG
GAT TAC GTC CTC AGC GTA TTC CCT CTC GCA GCT GGA GCC CGG CGC AAC

      2890            2900            2910            2920            2930
       •               •               •               •               •
CTG GCG TTT TTC CAT AGG CTC CGC CCC CCT GAC GAG CAT CAC AAA AAT
GAC CGC AAA AAG GTA TCC GAG GCG GGG GGA CTG CTC GTA GTG TTT TTA

         2940            2950            2960            2970            2980
          •               •               •               •               •
CGA CGC TCA AGT CAG AGG TGG CGA AAC CCG ACA GGA CTA TAA AGA TAC
GCT GCG AGT TCA GTC TCC ACC GCT TTG GGC TGT CCT GAT ATT TCT ATG
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FIG. 4 E

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      1930          1940          1950          1960          1970
       •             •             •             •             •
GAG GGG AAT GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC
CTC CCC TTA CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG
Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn>

         1980          1990          2000          2010          2020
          •             •             •             •             •
CAC TAC ACA CAG AAG AGC CTC TCC CTG TCT CTG GGT AAA T GAG TGC CAG
GTG ATG TGT GTC TTC TCG GAG AGG GAC AGA GAC CCA TTT A CTC ACG GTC
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Xxx>

      2030          2040          2050          2060          2070
       •             •             •             •             •
GGC CGG CAA GCC CCC GCT CCC CGG GCT CTC GGG GTC GCG CGA GGA TGC
CCG GCC GTT CGG GGG CGA GGG GCC CGA GAG CCC CAG CGC GCT CCT ACG

         2080          2090          2100          2110
          •             •             •             •
TTG GCA CGT ACC CCG TCT ACA TAC TTC CCA GGC ACC CAG CAT GGA AAT
AAC CGT GCA TGG GGC AGA TGT ATG AAG GGT CCG TGG GTC GTA CCT TTA

   2120          2130          2140          2150          2160
    •             •             •             •             •
AAA GCA CCC ACC ACT GCC CTG GGC CCC TGT GAG ACT GTG ATG GTT CTT
TTT CGT GGG TGG TGA CGG GAC CCG GGG ACA CTC TGA CAC TAC CAA GAA

      2170          2180          2190          2200          2210
       •             •             •             •             •
TCC ACG GGT CAG GCC GAG TCT GAG GCC TGA GTG ACA TGA GGG AGG CAG
AGG TGC CCA GTC CGG CTC AGA CTC CGG ACT CAC TGT ACT CCC TCC GTC

         2220          2230          2240          2250          2260
          •             •             •             •             •
AGC GGG TCC CAC TGT CCC CAC ACT GGC CCA GGC TGT GCA GGT GTG CCT
TCG CCC AGG GTG ACA GGG GTG TGA CCG GGT CCG ACA CGT CCA CAC GGA

         2270          2280          2290          2300          2310
          •             •             •             •             •
GGG CCA CCT AGG GTG GGG CTC AGC CAG GGG CTG CCC TCG GCA GGG TGG
CCC GGT GGA TCC CAC CCC GAG TCG GTC CCC GAC GGG AGC CGT CCC ACC

         2320          2330          2340          2350
          •             •             •             •
GGG ATT TGC CAG CGT GGC CCT CCC TCC AGC AGC AGG ACT CTA GAG GAT
CCC TAA ACG GTC GCA CCG GGA GGG AGG TCG TCG TCC TGA GAT CTC CTA

   2360          2370          2380          2390          2400
    •             •             •             •             •
CAT AAT CAG CCA TAC CAC ATT TGT AGA GGT TTT ACT TGC TTT AAA AAA
GTA TTA GTC GGT ATG GTG TAA ACA TCT CCA AAA TGA ACG AAA TTT TTT

      2410          2420          2430          2440          2450
       •             •             •             •             •
CCT CCC ACA CCT CCC CCT GAA CCT GAA ACA TAA AAT GAA TGC AAT TGT
GGA GGG TGT GGA GGG GGA CTT GGA CTT TGT ATT TTA CTT ACG TTA ACA
```

FIG. 4 D

```
            1450          1460          1470          1480
             •             •             •             •
AAG CCG CGG GAG GAG CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC
TTC GGC GCC CTC CTC GTC AAG TTG TCG TGC ATG GCA CAC CAG TCG CAG
Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val>

      1490          1500          1510          1520          1530
       •             •             •             •             •
CTC ACC GTC CTG CAC CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC
GAG TGG CAG GAC GTG GTC CTG ACC GAC TTG CCG TTC CTC ATG TTC ACG
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys>

      1540          1550          1560          1570          1580
       •             •             •             •             •
AAG GTC TCC AAC AAA GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC
TTC CAG AGG TTG TTT CCG GAG GGC AGG AGG TAG CTC TTT TGG TAG AGG
Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser>

      1590          1600          1610          1620          1630
       •             •             •             •             •
AAA GCC AAA GG TGG GAC CCA CGG GGT GCG AGG GCC ACA TGG ACA GAG GTC
TTT CGG TTT CC ACC CTG GGT GCC CCA CGC TCC CGG TGT ACC TGT CTC CAG
Lys Ala Lys>

      1640          1650          1660          1670          1680
       •             •             •             •             •
AGC TCG GCC CAC CCT CTG CCC TGG GAG TGA CCG CTG TGC CAA CCT CTG
TCG AGC CGG GTG GGA GAC GGG ACC CTC ACT GGC GAC ACG GTT GGA GAC

      1690          1700          1710          1720          1730
       •             •             •             •             •
TCC CTA CA GGG CAG CCC CGA GAG CCA CAG GTG TAC ACC CTG CCC CCA TCC
AGG GAT GT CCC GTC GGG GCT CTC GGT GTC CAC ATG TGG GAC GGG GGT AGG
           Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser>

      1740          1750          1760          1770          1780
       •             •             •             •             •
CAG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA
GTC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT
Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys>

      1790          1800          1810          1820
       •             •             •             •
GGC TTC TAC CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG
CCG AAG ATG GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln>

  1830          1840          1850          1860          1870
   •             •             •             •             •
CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC
GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly>

  1880          1890          1900          1910          1920
   •             •             •             •             •
TCC TTC TTC CTC TAC AGC AGG CTA ACC GTG GAC AAG AGC AGG TGG CAG
AGG AAG AAG GAG ATG TCG TCC GAT TGG CAC CTG TTC TCG TCC ACC GTC
Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln>
```

FIG. 4 C

```
         920           930           940           950           960
          •             •             •             •             •
GGC AGC CAC AGG CTG GAT GCC CCT ACC CCA GGC CCT GCG CAT ACA GGG
CCG TCG GTG TCC GAC CTA CGG GGA TGG GGT CCG GGA CGC GTA TGT CCC

               970           980           990          1000
                •             •             •             •
GCA GGT GCT GCG CTC AGA CCT GCC AAG AGC CAT ATC CGG GAG GAC CCT
CGT CCA CGA CGC GAG TCT GGA CGG TTC TCG GTA TAG GCC CTC CTG GGA

 1010         1020          1030          1040          1050
  •             •             •             •             •
GCC CCT GAC CTA AGC CCA CCC CAA AGG CCA AAC TCT CCA CTC CCT CAG
CGG GGA CTG GAT TCG GGT GGG GTT TCC GGT TTG AGA GGT GAG GGA GTC

    1060          1070          1080          1090          1100
     •             •             •             •             •
CTC AGA CAC CTT CTC TCC TCC CAG ATT CGA GTA ACT CCC AAT CTT CTC
GAG TCT GTG GAA GAG AGG AGG GTC TAA GCT CAT TGA GGG TTA GAA GAG

       1110          1120          1130          1140          1150
        •             •             •             •             •
TCT GCA GAG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA GGT AAG
AGA CGT CTC AGG TTT ATA CCA GGG GGT ACG GGT AGT ACG GGT CCA TTC
    Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro>

          1160          1170          1180          1190          1200
           •             •             •             •             •
CCA ACC CAG GCC TCG CCC TCC AGC TCA AGG CGG GAC AGG TGC CCT AGA
GGT TGG GTC CGG AGC GGG AGG TCG AGT TCC GCC CTG TCC ACG GGA TCT

             1210          1220          1230          1240
              •             •             •             •
GTA GCC TGC ATC CAG GGA CAG GCC CCA GCC GGG TGC TGA CGC ATC CAC
CAT CGG ACG TAG GTC CCT GTC CGG GGT CGG CCC ACG ACT GCG TAG GTG

 1250         1260          1270          1280          1290
  •             •             •             •             •
CTC CAT CTC TTC CTC AGC A CCT GAG TTC CTG GGG GGA CCA TCA GTC TTC
GAG GTA GAG AAG GAG TCG T GGA CTC AAG GAC CCC CCT GGT AGT CAG AAG
                        Pro Glu Phe Leu Gly Gly Pro Ser Val Phe>

 1300         1310          1320          1330          1340
  •             •             •             •             •
CTC TTC CCC CCA AAA CCC AAG GAC ACT CTC ATG ATC TCC CGG ACC CCT
GAC AAG GGG GGT TTT GGG TTC CTG TGA GAG TAC TAG AGG GCC TGG GGA
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro>

    1350          1360          1370          1380          1390
     •             •             •             •             •
GAG GTC ACG TGC GTG GTG GTG GAC GTG AGC CAG GAA GAC CCC GAG GTC
CTC CAG TGC ACG CAC CAC CAC CTG CAC TCG GTC CTT CTG GGG CTC CAG
Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val>

       1400          1410          1420          1430          1440
        •             •             •             •             •
CAG TTC AAC TGG TAC GTG GAT GGC GTG GAG GTG CAT AAT GCC AAG ACA
GTC AAG TTG ACC ATG CAC CTA CCG CAC CTC CAC GTA TTA CGG TTC TGT
Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr>
```

FIG. 4 B

```
              440           450           460           470           480
               •             •             •             •             •
AAG GGC CCA TCC GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC
TTC CCG GGT AGG CAG AAG GGG GAC CGC GGG ACG AGG TCC TCG TGG AGG
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser>


                    490           500           510           520
                     •             •             •             •
GAG AGC ACA GCC GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA
CTC TCG TGT CGG CGG GAC CCG ACG GAC CAG TTC CTG ATG AAG GGG CTT
Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu>


  530           540           550           560           570
   •             •             •             •             •
CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC
GGC CAC TGC CAC AGC ACC TTG AGT CCG CGG GAC TGG TCG CCG CAC GTG
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His>


     580           590           600           610           620
      •             •             •             •             •
ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC
TGG AAG GGC CGA CAG GAT GTC AGG AGT CCT GAG ATG AGG GAG TCG TCG
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser>


     630           640           650           660           670
      •             •             •             •             •
GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC
CAC CAC TGG CAC GGG AGG TCG TCG AAC CCG TGC TTC TGG ATG TGG ACG
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys>


     680           690           700           710           720
      •             •             •             •             •
AAC GTA GAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT GGT
TTG CAT CTA GTG TTC GGG TCG TTG TGG TTC CAC CTG TTC TCT CAA CCA
Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val>


              730           740           750           760
               •             •             •             •
GAG AGG CCA GCA CAG GGC AGG GAG GGT GTC TGC TGG AAG CCA GGC TCA
CTC TCC CGT CGT GTC CCG TCC CTC CCA CAG ACG ACC TTC GGT CCG AGT


  770           780           790           800           810
   •             •             •             •             •
GCC CTC CTC CCT GGA CGC ACC CCG GCT GTG CAG CCC CAG CCC AGG GCA
CGG GAG GAC GGA CCT GCG TGG GCG CGA CAC GTC GGG GTC GGG TCC CGT


     820           830           840           850           860
      •             •             •             •             •
GCA AGG CAT GCC CCA TCT GTC TCC TCA CCC GGA GGC CTC TGA CCA CCC
CGT TCC GTA CGG GGT AGA CAG AGG AGT GGG CCT CCG GAG ACT GGT GGG


     870           880           890           900           910
      •             •             •             •             •
CAC TCA TGC TCA GGG AGA GGG TCT TCT GGA TTT TTC CAC CAG GCT CCG
GTG AGT ACG AGT CCC TCT CCC AGA AGA CCT AAA AAG GTG GTC CGA GGC
```

FIG. 4 A The pEe6TF8HCDR20 expression vector DNA sequence. The coding regions of the TF8-5G9 CDR-grafted HC gene, TF8HCDR20, are translated.

Sequence Range: 1 to 7073

```
            10            20            30            40
             *             *             *             *
GAA TTC GCC GCC ACC ATG GAA TGG AGC TGG GTC TTT CTC TTC TTC TTG
CTT AAG CGG CGG TGG TAC CTT ACC TCG ACC CAG AAA GAG AAG AAG AAC
            Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu>

    50            60            70            80            90
     *             *             *             *             *
TCA GTA ACT ACA GGT GTA CAC TCA CAA GTT CAG CTG GTG GAG TCT GGA
AGT CAT TGA TGT CCA CAT GTG AGT GTT CAA GTC GAC CAC CTC AGA CCT
Ser Val Thr Thr Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly>

      100           110           120           130           140
       *             *             *             *             *
GGA GGA GTA GTA CAA CCT GGA AGG TCA CTG AGA CTG TCT TGT AAG GCT
CCT CCT CAT CAT GTT GGA CCT TCC AGT GAC TCT GAC AGA ACA TTC CGA
Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala>

      150           160           170           180           190
       *             *             *             *             *
AGT GGA TTC AAT ATC AAG GAC TAT TAT ATG CAC TGG GTC AGA CAA GCT
TCA CCT AAG TTA TAG TTC CTG ATA ATA TAC GTG ACC AGT CTT GTT CGA
Ser Gly Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala>

         200           210           220           230           240
          *             *             *             *             *
CCT GGA AAA GGA CTC GAG TGG ATA GGT TTA ATT GAT CCT GAG AAT GGT
GGA CCT TTT CCT GAG CTC ACC TAT CCA AAT TAA CTA GGA CTC TTA CCA
Pro Gly Lys Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly>

            250           260           270           280
             *             *             *             *
AAC ACG ATA TAT GAT CCC AAG TTC CAA GGA AGA TTC ACA ATT TCT GCA
TTG TGC TAT ATA CTA GGG TTC AAG GTT CCT TCT AAG TGT TAA AGA CGT
Asn Thr Ile Tyr Asp Pro Lys Phe Gln Gly Arg Phe Thr Ile Ser Ala>

290           300           310           320           330
 *             *             *             *             *
GAC AAC TCT AAG AAT ACA CTG TTC CTG CAG ATG GAC TCA CTC AGA CCT
CTG TTG AGA TTC TTA TGT GAC AAG GAC GTC TAC CTG AGT GAG TCT GGA
Asp Asn Ser Lys Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro>

   340           350           360           370           380
    *             *             *             *             *
GAG GAT ACA GCA GTC TAC TAT TGT GCT AGA GAT AAC AGT TAT TAC TTC
CTC CTA TGT CGT CAG ATG ATA ACA CGA TCT CTA TTG TCA ATA ATG AAG
Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe>

      390           400           410           420           430
       *             *             *             *             *
GAC TAC TGG GGC CAA GGA ACA CCA GTC ACC GTG AGC TCA GCT TCC ACC
CTG ATG ACC CCG GTT CCT TGT GGT CAG TGG CAC TCG AGT CGA AGG TGG
Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr>
```
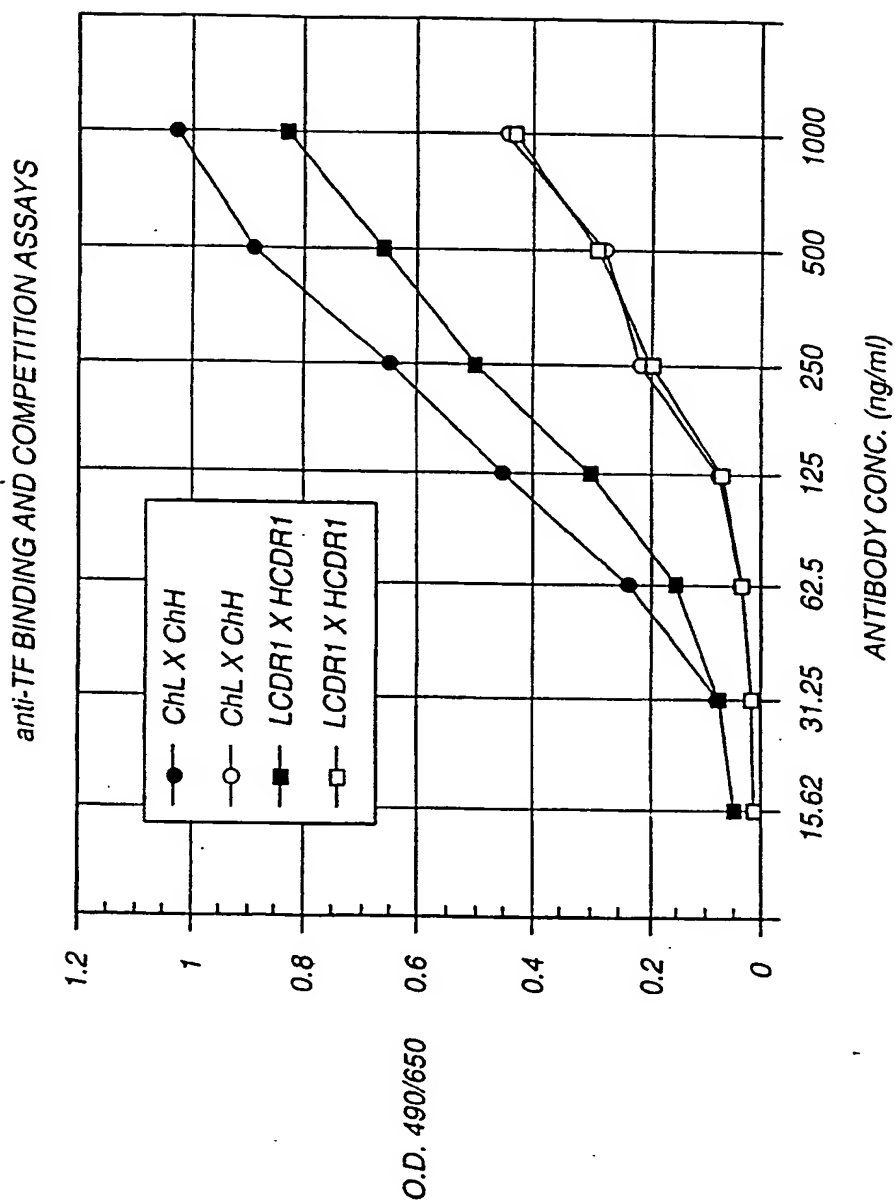
FIG. 3

anti-TF BINDING AND COMPETITION ASSAYS

●	ChL X ChH
○	ChL X ChH
■	LCDR1 X HCDR1
□	LCDR1 X HCDR1

O.D. 490/650

ANTIBODY CONC. (ng/ml)

FIG. 2 C

```
     820              830              840              850              860
      •                •                •                •                •
ACC TCC TCC CCA CCT CCT TCT CCT CCT CCT CCC TTT CCT TGG CTT TTA
TGG AGG AGG GGT GGA GGA AGA GGA GGA GGA GGG AAA GGA ACC GAA AAT

     870              880              890              900              910
      •                •                •                •                •
TCA TGC TAA TAT TTG CAG AAA ATA TTC AAT AAA GTG AGT CTT TGC ACT
AGT ACG ATT ATA AAC GTC TTT TAT AAG TTA TTT CAC TCA GAA ACG TGA

     920              930
      •                •
TGA AAA AAA AAA AAA AAA AAA A
ACT TTT TTT TTT TTT TTT TTT T
```

FIG. 2B

```
    340          350          360          370          380
     •            •            •            •            •
GGT GAG AGC CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAC
CCA CTC TCG GGC ATG TGC AAG CCT CCC CCC TGG TTC GAC CTT TAT TTG
Gly Glu Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn>

    390          400          410          420          430
     •            •            •            •            •
AGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA TCC AGT GAG
TCC CGA CTA CGA CGT GGT TGA CAT AGG TAG AAG GGT GGT AGG TCA CTC
Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu>

       440          450          460          470          480
        •            •            •            •            •
CAG TTA ACA TCT GGA GGT GCC TCA GTC GTG TGC TTC TTG AAC AAC TTC
GTC AAT TGT AGA CCT CCA CGG AGT CAG CAC ACG AAG AAC TTG TTG AAG
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe>

       490          500          510          520
        •            •            •            •
TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT GAT GGC AGT GAA CGA
ATG GGG TTT CTG TAG TTA CAG TTC ACC TTC TAA CTA CCG TCA CTT GCT
Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg>

    530          540          550          560          570
     •            •            •            •            •
CAA AAT GGC GTC CTG AAC AGT TGG ACT GAT CAG GAC AGC AAA GAC AGC
GTT TTA CCG CAG GAC TTG TCA ACC TGA CTA GTC CTG TCG TTT CTG TCG
Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser>

    580          590          600          610          620
     •            •            •            •            •
ACC TAC AGC ATG AGC AGC ACC CTC ACG TTG ACC AAG GAC GAG TAT GAA
TGG ATG TCG TAC TCG TCG TGG GAG TGC AAC TGG TTC CTG CTC ATA CTT
Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu>

    630          640          650          660          670
     •            •            •            •            •
CGA CAT AAC AGC TAT ACC TGT GAG GCC ACT CAC AAG ACA TCA ACT TCA
GCT GTA TTG TCG ATA TGG ACA CTC CGG TGA GTG TTC TGT AGT TGA AGT
Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser>

       680          690          700          710          720
        •            •            •            •            •
CCC ATT GTC AAG AGC TTC AAC AGG AAT GAG TGT TA GAG ACA AAG GTC CTG
GGG TAA CAG TTC TCG AAG TTG TCC TTA CTC ACA AT CTC TGT TTC CAG GAC
Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys>

       730          740          750          760          770
        •            •            •            •            •
AGA CGC CAC CAC CAG CTC CCC AGC TCC ATC CTA TCT TCC CTT CTA AGG
TCT GCG GTG GTG GTC GAG GGG TCG AGG TAG GAT AGA AGG GAA GAT TCC

       780          790          800          810
        •            •            •            •
TCT TGG AGG CTT CCC CAC AAG CGA CCT ACC ACT GTT GCG GTG CTC CAA
AGA ACC TCC GAA GGG GTG TTC GCT GGA TGG TCA CAA CGC CAC GAG GTT
```

RECTIFIED SHEET (RULE 91)
ISA/EP

Sequence of the murine TF8-5G9 light chain cDNA with protein translation. The essential regions of the cDNA are as follows:

FIG. 2 A

Nucleotides	Region
1-4	5' untranslated.
5-64	Start codon and leader sequence.
65-385	Variable region.
386-706	Murine kappa constant region.
707-917	3' untranslated region.
918-937	Poly A tail.

Sequence Range: 1 to 937

```
                10              20              30              40
                 •               •               •               •
      GGA C ATG CGG GCC CCT GCT CAG TTT TTT GGG ATC TTG TTG CTC TGG TTT
      CCT G TAC GCC CGG GGA CGA GTC AAA AAA CCC TAG AAC AAC GAG ACC AAA
            Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe>

      50              60              70              80              90
       •               •               •               •               •
      CCA GGT ATC AGA TGT GAC ATC AAG ATG ACC CAG TCT CCA TCC TCC ATG
      GGT CCA TAG TCT ACA CTG TAG TTC TAC TGG GTC AGA GGT AGG AGG TAC
      Pro Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met>

      100             110             120             130             140
       •               •               •               •               •
      TAT GCA TCG CTG GGA GAG AGA GTC ACT ATC ACT TGT AAG GCG AGT CAG
      ATA CGT AGC GAC CCT CTC TCT CAG TGA TAG TGA ACA TTC CGC TCA GTC
      Tyr Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln>

      150             160             170             180             190
       •               •               •               •               •
      GAC ATT AGA AAG TAT TTA AAC TGG TAC CAG CAG AAA CCA TGG AAA TCT
      CTG TAA TCT TTC ATA AAT TTG ACC ATG GTC GTC TTT GGT ACC TTT AGA
      Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser>

      200             210             220             230             240
       •               •               •               •               •
      CCT AAG ACC CTG ATC TAT TAT GCA ACA AGC TTG GCA GAT GGG GTC CCA
      GGA TTC TGG GAC TAG ATA ATA CGT TGT TCG AAC CGT CTA CCC CAG GGT
      Pro Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro>

      250             260             270             280
       •               •               •               •
      TCA AGA TTC AGT GGC AGT GGA TCT GGG CAA GAT TAT TCT CTA ACC ATC
      AGT TCT AAG TCA CCG TCA CCT AGA CCC GTT CTA ATA AGA GAT TGG TAG
      Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile>

      290     300             310             320             330
       •       •               •               •               •
      AGC AGC CTG GAG TCT GAC GAT ACA GCA ACT TAT TAC TGT CTA CAA CAT
      TCG TCG GAC CTC AGA CTG CTA TGT CGT TGA ATA ATG ACA GAT GTT GTA
      Ser Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His>
```

FIG. 1 D

```
      1300            1310            1320            1330            1340
       •               •               •               •               •
  TGG GAG GCA GGA AAT ACT TTC ACC TGC TCT GTG TTA CAT GAG GGC CTG
  ACC CTC CGT CCT TTA TGA AAG TGG ACG AGA CAC AAT GTA CTC CCG GAC
  Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu>


      1350            1360            1370            1380            1390
       •               •               •               •               •
  CAC AAC CAC CAT ACT GAG AAG AGC CTC TCC CAC TCT CCT GGT AAA TG ATC
  GTG TTG GTG GTA TGA CTC TTC TCG GAG AGG GTG AGA GGA CCA TTT AC TAG
  His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys>


      1400            1410            1420            1430            1440
       •               •               •               •               •
  CCA GTG TCC TTG GAG CCC TCT GGT CCT ACA GGA CTC TGA CAC CTA CCT
  GGT CAC AGG AAC CTC GGG AGA CCA GGA TGT CCT GAG ACT GTG GAT GGA


      1450            1460            1470            1480
       •               •               •               •
  CCA CCC CTC CCT GTA TAA ATA AAG CAC CCA GCA CTG CCT TGG ACC C
  GGT GGG GAG GGA CAT ATT TAT TTC GTG GGT CGT GAC GGA ACC TGG G .
```

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FIG. 1 C

```
      820         830         840         850         860
       •           •           •           •           •
CCT AAG GTC ACG TGT GTT GTG GTA GAC ATC AGC AAG GAT GAT CCC CAG
GGA TTC CAG TGC ACA CAA CAC CAT CTG TAG TCG TTC CTA CTA GGG CTC
Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu>

      870         880         890         900         910
       •           •           •           •           •
GTC CAG TTC AGC TGG TTT GTA GAT GAT GTG GAG GTG CAC ACA GCT CAG
CAG GTC AAG TCG ACC AAA CAT CTA CTA CAC CTC CAC GTG TGT CGA GTC
Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln>

      920         930         940         950         960
       •           •           •           •           •
ACG CAA CCC CGG GAG GAG CAG TTC AAC AGC ACT TTC CGC TCA GTC AGT
TGC GTT GGG GCC CTC CTC GTC AAG TTG TCG TGA AAG GCG AGT CAG TCA
Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser>

      970         980         990        1000
       •           •           •           •
GAA CTT CCC ATC ATG CAC CAG GAC TGG CTC AAT GGC AAG GAG TTC AAA
CTT GAA GGG TAG TAC GTG GTC CTG ACC GAG TTA CCG TTC CTC AAG TTT
Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys>

1010       1020        1030        1040        1050
  •          •           •           •           •
TGC AGG GTC AAC AGT GCA GCT TTC CCT GCC CCC ATC GAG AAA ACC ATC
ACG TCC CAG TTG TCA CGT CGA AAG GCA CGG GGG TAG CTC TTT TGG TAG
Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile>

1060        1070        1080        1090        1100
  •           •           •           •           •
TCC AAA ACC AAA GGC AGA CCG AAG GCT CCA CAG GTG TAC ACC ATT CCA
AGG TTT TGG TTT CCG TCT GGC TTC CGA GGT GTC CAC ATG TGG TAA GGT
Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro>

   1110        1120        1130        1140      . 1150
     •           •           •           •           •
CCT CCC AAG GAG CAG ATG GCC AAG GAT AAA GTC AGT CTG ACC TGC ATG
GGA GGG TTC CTC GTC TAC CGG TTC CTA TTT CAG TCA GAC TGG ACG TAC
Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met>

   1160        1170        1180        1190        1200
     •           •           •           •           •
ATA ACA GAC TTC TTC CCT GAA GAC ATT ACT GTC GAG TGG CAG TGG AAT
TAT TGT CTG AAG AAG GGA CTT CTG TAA TGA CAC CTC ACC GTC ACC TTA
Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn>

   1210        1220        1230        1240
     •           •           •           •
CGG CAG CCA GCG GAG AAC TAC AAG AAC ACT CAG CCC ATC ATG GAC ACA
CCC GTC GGT CGC CTC TTG ATG TTC TTG TGA GTC GGG TAG TAC CTG TGT
Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr>

1250        1260        1270        1280        1290
  •           •           •           •           •
GAT GGC TCT TAC TTC GTC TAC AGC AAG CTC AAT GTG CAG AAG AGC AAC
CTA CCG AGA ATG AAG CAG ATG TCG TTC GAG TTA CAC GTC TTC TCG TTG
Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn>
```

FIG. 1 B

```
        340           350           360           370           380
         •             •             •             •             •
ACT·GCC GTC TAT TAC TGT GCT AGA GAT AAC TCG TAC TAC TTT GAC TAC
TGA CGG CAG ATA ATG ACA CGA TCT CTA TTG AGC ATG ATG AAA CTG ATG
Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr>

        390           400           410           420           430
         •             •             •             •             •
TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC AAA ACG ACA CCC
ACC CCG GTT CCG TGG TGA GAG TGT CAG AGG AGT CGG TTT TGC TGT GGG
Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro>

        440           450           460           470           480
         •             •             •             •             •
CCA TCT GTC TAT CCA CTG GCC CCT GGA TCT GCT GCC CAA ACT AAC TCC
GGT AGA CAG ATA GGT GAC CGG GGA CCT AGA CGA CGG GTT TGA TTG AGG
Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser>

        490           500           510           520
         •             •             •             •
ATG GTG ACC CTG GGA TGC CTG GTC AAG GGC TAT TTC CCT GAG CCA GTG
TAC CAC TGG GAC CCT ACG GAC CAG TTC CCG ATA AAG GGA CTC GGT CAC
Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val>

530          540           550           560           570
 •            •             •             •             •
ACA GTG ACC TGG AAC TCT GGA TCC CTG TCC AGC GGT GTG CAC ACC TTC
TGT CAC TGG ACC TTG AGA CCT AGG GAC AGG TCG CCA CAC GTG TGG AAG
·Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe>

··580          590           600           610           620
 •            •             •             •             •
CCA GCT GTC CTG CAG TCT GAC CTC TAC ACT CTG AGC AGC TCA GTG ACT
GGT CGA CAG GAC GTC AGA CTG GAG ATG TGA GAC TCG TCG AGT CAC TGA
Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr>

        630           640           650           660           670
         •             •             •             •             •
GTC CCC TCC AGC ACC TGG CCC AGC GAG ACC GTC ACC TGC AAC GTT GCC
CAG GGG AGG TCG TGG ACC GGG TCG CTC TGG CAG TGG ACG TTG CAA CGG
Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala>

        680           690           700           710           720
         •             •             •             •             •
CAC CCG GCC AGC AGC ACC AAG GTG GAC AAG AAA ATT GTG CCC AGG GAT
GTG GGC CGG TCG TCG TGG TTC CAC CTG TTC·TTT TAA CAC GGG TCC CTA
His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp>

        730           740           750           760
         •             •             •             •
TGT GGT TGT AAG CCT TGC ATA TGT ACA GTC CCA GAA GTA TCA TCT GTC
ACA CCA ACA TTC GGA ACG TAT ACA TGT CAG GGT CTT CAT AGT AGA CAG
Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val>

770          780           790           800           810
 •            •             •             •             •
TTC ATC TTC CCC CCA AAG CCC AAG GAT GTG CTC ACC ATT ACT CTG ACT
AAG TAG AAG GGG GGT TTC GGG TTC CTA CAC GAG TGG TAA TGA GAC TGA
Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr>
```

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ISA/EP

1/41

Sequence of the murine TF8-5G9 heavy chain cDNA with protein translation. The essential regions of the cDNA are as follows:

FIG. 1 A

Nucleotides	Region
1-10	5' untranslated region.
11-67	Start codon and leader sequence.
68-418	Variable region.
419-1390	Murine IgG1 constant region.
1391-1489	3' untranslated region.

Sequence Range: 1 to 1489

```
            10              20              30              40
            •               •               •               •
GGT CCT TAC A ATG AAA TGC AGC TGG GTC ATC TTC TTC CTG ATG GCA GTG
CCA GGA ATG T TAC TTT ACG TCG ACC CAG TAG AAG AAG GAC TAC CGT CAC
            Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val>

   50              60              70              80              90
   •               •               •               •               •
GTT ACA GGG GTC AAT TCA GAG ATT CAG CTG CAG CAG TCT GGG GCT GAG
CAA TGT CCC CAG TTA AGT CTC TAA GTC GAC GTC GTC AGA CCC CGA CTC
Val Thr Gly Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu>

  100             110             120             130             140
   •               •               •               •               •
CTT GTG AGG CCA GGG GCC TTA GTC AAG TTG TCC TGC AAA GCT TCT GGC
GAA CAC TCC GGT CCC CGG AAT CAG TTC AAC AGG ACG TTT CGA AGA CCG
Leu Val Arg Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly>

     150             160             170             180             190
      •               •               •               •               •
TTC AAC ATT AAA GAC TAC TAT ATG CAC TGG GTG AAG CAG AGG CCT GAA
AAG TTG TAA TTT CTG ATG ATA TAC GTG ACC CAC TTC GTC TCC GGA CTT
Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu>

      200             210             220             230             240
       •               •               •               •               •
CAG GGC CTC GAG TCG ATT GGA TTG ATT GAT CCT GAG AAT GGT AAT ACT
GTC CCG GAC CTC ACC TAA CCT AAC TAA CTA GGA CTC TTA CCA TTA TGA
Gln Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr>

         250             260             270             280
          •               •               •               •
ATA TAT GAC CCG AAG TTC CAG GGC AAG GCC AGT ATA ACA GCA GAC ACA
TAT ATA CTG GGC TTC AAG GTC CCG TTC CGG TCA TAT TGT CGT CTG TGT
Ile Tyr Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr>

  290       300             310             320             330
   •          •               •               •               •
TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT GAG GAC
AGG AGG TTG TGT CGG ATG GAC GTC GAG TCG TCG GAC TGT AGA CTC CTG
Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp>
```

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37.   The pharmaceutical composition of Claim
1 36 wherein said CDR-grafted antibody is TF8HCDR20 x
   TF8LCDR3.

5

10

15

20

25

30

35

26. The method of Claim 19 wherein said
1 expression vector comprising a nucleic acid encoding the
CDR-grafted antibody light chain is pEe12TF8LCDR3.
27. A nucleic acid encoding the heavy chain
of the CDR-grafted antibody of Claim 1.
5 28. A nucleic acid encoding the light chain
of the CDR-grafted antibody of Claim 1.
29. The nucleic acid of Claim 27 having the
sequence of nucleotides 1-2360 of SEQ ID NO:15.
30. The nucleic acid of Claim 28 having the
10 sequence of nucleotides 1-759 of SEQ ID NO:17.
31. A method of attenuation of coagulation
comprising administering a therapeutically effective
amount of a CDR-grafted antibody capable of inhibiting
human tissue factor to a patient in need of said
15 attenuation.
32. The method of Claim 31 wherein said CDR-
grafted antibody is TF8HCDR20 x TF84CDR3.
33. A method of treatment or prevention of
thrombotic disorder comprising administering a
20 therapeutically effective amount of a CDR-grafted
antibody capable of inhibiting human tissue factor to a
patient in need of said treatment or prevention.
34. The method of Claim 33 wherein said
thrombotic disorder is intravascular coagulation,
25 arterial restenosis or arteriosclerosis.
35. The method of Claim 33 or 34 wherein said
CDR-grafted antibody is TF8HCDR20 x TF8LCDR3.
36. A pharmaceutical composition comprising
at least one CDR-grafted antibody capable of inhibiting
30 human tissue factor and a pharmaceutically acceptable
carrier.

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18. The fragment of Claim 17 wherein said
1 fragment is an Fab or F(ab')₂ fragment.
19. A method of making the CDR-grafted
antibody of Claim 1 comprising cotransfecting a host
cell with an expression vector comprising a nucleic acid
5 encoding the CDR-grafted antibody heavy chain and an
expression vector comprising a nucleic acid encoding the
CDR-grafted antibody light chain; culturing the
transfected host cell; and recovering said CDR-grafted
antibody.
10   20. A method of making the CDR-grafted
antibody of Claim 1 comprising transfecting a host cell
with an expression vector comprising a nucleic acid
encoding the CDR-grafted antibody heavy chain and a
nucleic acid encoding the CDR-grafted antibody light
15 chain; culturing the transfected host cell; and
recovering said CDR-grafted antibody.
21. The method of Claim 18 or 19 wherein said
nucleic acid encoding the CDR-grafted antibody heavy
chain has the sequence of nucleotides 1-2360 of SEQ ID
20 NO:15.
22. The method of Claim 18 or 19 wherein said
nucleic acid encoding the CDR-grafted light chain has
the sequence of nucleotides 1-759 of SEQ ID NO:17.
23. The method of Claim 19 or 20 wherein said
25 host cell is a bacterial cell, yeast cell, insect cell
or mammalian cell.
24. The method of Claim 23 wherein said
mammalian cell is a CHO cell, COS cell or myeloma cell.
25. The method of Claim 19 wherein said
30 expression vector comprising a nucleic acid encoding the
CDR-grafted antibody heavy chain is pEe6TF8HCDR20.

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7.   The CDR-grafted antibody of Claim 1
1 wherein the heavy chain variable region has the amino
acid sequence of SEQ ID NO:11.

8.   The CDR-grafted antibody of Claim 1 or 7
wherein the light chain variable region has the amino
5 acid sequence of SEQ ID NO:12.

9.   The CDR-grafted antibody of Claim 1
wherein the heavy chain variable region has the amino
acid sequence of SEQ ID NO:13.

10.   The CDR-grafted antibody of Claim 1 or 9
10 wherein the light chain variable region has the amino
acid sequence of SEQ ID NO:14.

11.   The CDR-grafted antibody of Claim 1
wherein the heavy chain constant region is the human
IgG4 constant region.

15   12.   The CDR-grafted antibody of Claim 10
wherein the heavy chain constant region is the human
IgG4 constant region.

13.   The CDR-grafted antibody of Claim 1
wherein the light chain constant region is the human
20 kappa constant region.

14.   The CDR-grafted antibody of Claim 10
wherein the light chain constant region is the human
kappa constant region.

15.   CDR-grafted monoclonal antibody TF8HCDR1
25 x TF8LCDR1.

16.   CDR-grafted monoclonal antibody TF8HCDR20
x TF8LCDR3.

17.   A fragment of the CDR-grafted antibody of
Claim 1 wherein said fragment is capable of inhibiting
30 human tissue factor.

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WHAT IS CLAIMED IS:

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      1.   A CDR-grafted antibody capable of
inhibiting human tissue factor wherein the
complementarity determining regions (CDRs) are derived

5 from a non-human monoclonal antibody against tissue
factor and the framework (FR) and constant (C) regions
are derived from one or more human antibodies.

      2.   The CDR-grafted antibody of Claim 1
wherein said non-human monoclonal antibody is a murine

10 antibody.

      3.   The CDR-grafted antibody of Claim 2
wherein said murine antibody is TF8-5G9.

      4.   The CDR-grafted antibody of Claim 1
wherein said CDRs of the heavy chain have the amino acid

15 sequences:

CDR1	DDYMH	(SEQ ID NO:5)
CDR2	LIDPENGNTIYDPKFQG	(SEQ ID NO:6)
CDR3	DNSYYFDY	(SEQ ID NO:7)

and said CDRs of the light chain have the amino acid

20 sequences:

CDR1	KASQDIRKYLN	(SEQ ID NO:8)
CDR2	YATSLAD	(SEQ ID NO:9)
CDR3	LQHGESPYT	(SEQ ID NO:10).

      5.   The CDR-grafted antibody of Claim 1
25 wherein the FR of the heavy chain is derived from the
human antibody KOL.

      6.   The CDR-grafted antibody of Claim 1
wherein the FR of the light chain is derived from the
human antibody REI.

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    GACCGATCCA GCCTCCGCGG CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG    6960
 1  AGTGACGTAA GTACCGCCTA TAGAGTCTAT AGGCCCACCC CCTTGGCTTC TTATGCATGC    7020
    TATACTGTTT TTGGCTTCGG GTCTATACAC CCCCGCTTCC TCATGTTATA GGTGATGGTA    7080
    TAGCTTAGCC TATAGGTGTG GGTTATTGAC CATTATTGAC CACTCCCCTA TTGGTGACGA    7140
 5  TACTTTCCAT TACTAATCCA TAACATGGCT CTTTGCCACA ACTCTCTTTA TTGGCTATAT    7200
    GCCAATACAC TGTCCTTCAG AGACTGACAC GGACTCTGTA TTTTTACAGG ATGGGGTCTC    7260
    ATTTATTATT TACAAATTCA CATATACAAC ACCACCGTCC CCAGTGCCCG CAGTTTTTAT    7320
    TAAACATAAC GTGGGATCTC CACGCGAATC TCGGGTACGT GTTCCGGACA TGGGCTCTTC    7380
    TCCGGTAGCG GCGGAGCTTC TACATCCGAG CCCTGCTCCC ATGCCTCCAG CGACTCATGG    7440
10  TCGCTCGGCA TCTCCTTGCT CCTAACAGTG GAGGCCAGAC TTAGGCACAG CACGATGCCC    7500
    ACCACCACCA GTGTGCCGCA CAAGGCCGTG GCGGTAGGGT ATGTGTCTGA AAATGAGCTC    7560
    GGGGAGCGGG CTTGCACCGC TGACGCATTT GGAAGACTTA AGGCAGCGGC AGAAGAAGAT    7620
    GCAGGCAGCT GAGTTGTTGT GTTCTGATAA GAGTCAGAGG TAACTCCCGT TGCGGTGCTG    7680
15  TTAACGGTGG AGGGCAGTGT AGTCTGAGCA GTACTCGTTG CTGCCGCGCG CGCCACCAGA    7740
    CATAATAGCT GACAGACTAA CAGACTGTTC CTTTCCATGG GTCTTTTCTG CAGTCACCGT    7800
    CCTTGACACG AAGCTTGGGC TGCAGGTCGA TCGACTCTAG AGGATCGATC CCCGGGCGAG    7860
    CTCG                                                                7864
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